工學博士 學位論文

Bioelectrochemical Anaerobic Digestion for High-Rate Methane Production

고효율 메탄생산을 위한 생물전기화학 혐기성소화

指導敎授 宋 永 彩

2017年 8月

韓國海洋大學校 大學院

土 木 環 境 工 學 科

馮 慶

本 論文을 馮慶의 工學博士 學位論文으로 認准함.

委員長　　金 仁 洙　　(印)

委　員　　高 星 澈　　(印)

委　員　　安 容 台　　(印)

委　員　　蔡 奎 正　　(印)

委　員　　宋 永 彩　　(印)

2017年　6月

韓國海洋大學校 大學院
土木環境工學科 環境工學專攻
馮　慶

Contents

List of Tables

List of Figures

Bioelectrochemical Anaerobic Digestion for High-Rate Methane Production

QING FENG

Department of Civil and Environmental Engineering
Korea Maritime and Ocean University

Abstract

고농도의 유기오염물질을 안정화시키는 동시에 메탄가스를 회수할 수 있는 혐기성소화는 지난 100여년 이상동안 많은 연구자들에 의하여 연구되어 온 전통기술이며, 최근 지구온난화 문제가 전 세계적인 이슈로 급부상하면서 새롭게 조명을 받고 있다. 그러나 혐기성소화기술은 메탄생성균의 느린 성장속도와 환경인자에 대해 민감하여 상대적으로 긴 체류시간이 필요하고 유기물감량율이 낮으며, 운전조건이 까다롭다는 단점을 지니고 있다. 최근 들어 환경생물전기화학자들에 의해 연구되기 시작한 생물전기화학기술(Bioelectrochemical Technology)을 혐기성소화공정에 활용하면 전통적인 혐기성소화기술의 단점을 상당 부분 극복 가능한 것으로 연구되고 있다. 혐기성소화공정에 적용한 생물전기화학기술은 소화조 내에 설치하는 산화전극과 환원전극으로 이루어지며, 산화전극과 환원전극 사이에 외부회로를 구성하고 일정한 전위차가 유지되도록 외부전원을 이용하여 전압을 인가한 미생물전해전지(Microbial Electrolysis Cell, MEC)의 형태이다. 생물전기화학 혐기성소화조(Bioelectrochemical Anaerobic Digestion, BEAD)에서 전기적으로 활성을

가진 미생물들은 유기물이나 유기산을 빠른 속도로 분해하여 전자를 산화전극으로 공급하며, 환원전극의 표면에서는 수소나 메탄과 같은 바이오가스 생성반응이 일어나게 되는데 산화전극 및 환원전극에서의 반응은 인가전압에 의해서 촉진된다. 최근 들어 BEAD공정의 관심을 갖기 시작하여 활발히 연구되고 있다. 그러나 지금까지의 BEAD공정에 대한 연구 중에서 산화/환원전극 연구, 소화조 형태, 운전인자 및 실폐수/폐기물 처리 등에 관한 연구가 부족하다. 따라서 본 연구에서는 BEAD용 전극에 대한 연구를 하였으며, 교반형 BEAD를 설계하여 하수슬러지처리의 소화성능을 평가하였다. 또한, BEAD 소화조에서 메탄생성을 위한 전자전달 경로를 탐구하였으며, 상향류식 BEAD소화조를 설계하여 산성 주정폐수를 처리 성능을 평가하였다.

BEAD용 산화전극은 흑연직물섬유(Graphite Fiber Fabric, GFF)의 표면에 몇 가지의 방법으로 다중벽탄소나노튜브 (Multi-wall Carbon Nanotube, MWCNT), 팽창흑연 (Exfoliated Graphite, EG) 및 니켈을 고정하여 회분식 소화조에서 소화성능을 평가하였다. 이 중에 MWCNT와 니켈을 혼합한 전해질에서 GFF 표면을 전기영동전착법으로 표면처리한 후 콜타르 피치결합제로 제작한 산화전극은 최대메탄발생율과 메탄발생수율이 각각 47.4 mL CH_4/g COD.d 및 322.9 mL CH_4/g COD_r로 가장 높게 평가되었으며, 최종메탄발생량은 전극을 설치하지 않은 대조구에 비해 약 151% 증가하였다. 또한, 전기영동전착법으로 MWCNT와 Ni촉매를 고정한 환원전극은 최대메탄발생율과 메탄수율이 각각 44.8 mL CH_4/g COD.d 및 326.3mL CH_4/g COD_r로 가장 높았으며, 최종메탄발생량은 Ammonia촉매, FePc촉매 그리고 촉매대조구보다 약 32-52% 증가하였다. 이에 따라 Ni이 생물전기화학반응에 의한 이산화탄소의 메탄환원반응에 가장 우수한 촉매로 평가되었다.

하수슬러지를 처리하기 위해 유효부피 12L인 교반형 BEAD소화조를 제작하여 수리학적 체류시간(Hydraulic Retention Time, HRT), 온도 및 인가전압이 소화성능에 미친 영향을 평가하였다. 메탄가스 발생량은 HRT의 감소에 따라 증가한 부하율로 인하여 점차 증가하였으며, 메탄함량은 HRT 15~20일에서 약 77%으로 HRT 5~10일의 75%보다 약간 높았다. VS 감량은 HRT 20일에서는 70%로 대단히 높은 값을 보였으며,

HRT의 감소에 따라 점차 감소하였으나 HRT 5일에서도 약 52%의 높은 값으로 HRT 20일의 재래식 혐기성소화효율과 비슷하였다. 상온조건(25 ℃)에서 BEAD공정의 메탄가스 발생량은 중온조건(35 ℃)에 비하여 약 12.3% 감소하였으나, VS 감량은 비슷한 것으로 평가되었다. 그러나 바이오가스의 메탄함량과 공정의 에너지효율은 큰 차이가 없으며, 상당한양의 가열 에너지를 절약할 수 있는 것으로 판단되었다. 또한, 상온조건에서 BEAD공정을 산화전극과 환원전극 사이에 0.3 ~ 0.7V의 전위차를 유지하여 소화성능을 평가하였다. 전위차가 0.3 ~ 0.5V로 인가하였을 때에서 높은 소화효율을 비슷하게 유지하였으며, 0.3V에서 가장 높은 메탄발생량 (370 ml CH_4/L.d)을 보였고 0.5V에서 가장 높은 메탄함량(80.6%)으로 나타내었다. 그러나 전위차가 0.7V로 증가하는 경우 고농도의 휘발성지방산 (Volatile fatty acid, VFA)이 축적되어 소화효율은 급격히 감소하였다. BEAD의 부유 혐기성미생물종은 산화전극과 환원전극의 전위차 0.3V, 0.5V에서 혐기성미생물인 *Cloacamonas sp.*가 우점하였고, 전위차 0.7V에서는 가수분해미생물인 *Saprospiraceae sp., Fimbriimonas sp., Ottowia pentelensis*의 비율이 높았다. 이에 따라 바이오가스의 메탄함량과 메탄수율이 측면에서는 산화전극과 환원전극의 전위차를 0.5V로 인가하여 운전하는 것이 유리하며, 비메탄발생량과 에너지효율 측면에서는 전위차를 0.3V로 운전하는 것이 유리한 것으로 판단되었다.

지금까지 재래식혐기성소화조에서 메탄이 발생하는 주요경로를 초산, 수소/개미산을 경유하는 간접전자전달(Indirect Interspecies Electron Transfer, IIET) 경로로 설명하여 왔다. 그러나, BEAD소화조에서는 전기활성미생물과 메탄생성균 사이의 직접종간전자전달(Direct Interspecies Electron Transfer, DIET)이 메탄발생에 큰 역할을 할 수 있다. 이에 따라 BEAD소화조에 생장하는 전기활성미생물 및 부유혐기성미생물의 상호관계를 탐구하였으며 메탄생성과 관련한 전자전달경로를 아래와 같이 발견하였다. 산화전극 및 환원전극에 통해 발생하는 메탄은 전극을 매개로한 직접종간전자전달(Direct Interspecies Electron Transfer via electrode, eDIET)로 정의한다. 한편, DIET는 근접거리에 존재하는 전기활성미생물과 메탄균 사이에서 일어나는 생물학적 종간직접전자전달

(biological Direct Interspecies Electron Transfer, bDIET)과 소화조에 활성탄, 마그네타이트와 같은 전도성물질이 존재하는 경우 이러한 전도성물질을 매개로한 전자전달(Direct Interspecies Electron Transfer via conductive material, cDIET) 반응에 의해서 일어나기도 한다. 또한, 전기활성미생물이 내생상태에서 분비하는 플래빈 등의 산화환원물질(Shuttle)은 간접종간전자전달을 매개하는 sIIET 반응에 관여하며, 초산, 수소/개미산 등 중간체(intermediates)을 경유하는 간접종간전자전달은 iIIET로 구별되었다. 이에 따라 BEAD 소화조에서 전기활성미생물의 인해 bDIET를 위주로 여러 가지의 전자전달경로(eDIET, cDIET, sIIET, iIIET)를 통해 메탄발생량 및 유기물 감량율 등 소화효율을 향상시키는 것으로 판단하였다.

또한, 기존 상향류식 혐기성 슬러지 블랭킷 (Upflow Anaerobic Sludge Blanket, UASB)에 BEAD공정의 원리를 이용하여 상향류식 혐기성 생물전기화학 혐기성소화(Upflow Anaerobic Bioelectrochemical, UABE)공정을 개발하였으며, 산성 주정폐수를 처리하는 소화성능을 평가하였다. UABE 소화조의 최적 유입 pH는 5.6-7.0이었으며, 최적 유출수의 순환율이 유입수의 1.5-3.0배이었을 때 소화성능이 가장 높게 나타내었다. 특히, 주정폐수 유기물부하율 8g COD/L.d에서 유출수의 순환율 3.0로 운전한 UABE 반응조의 메탄발생량은 3,800 mL/L.d로 가장 높은 값을 나타내었으며, 동일한 조건에서 운전한 UASB 반응조의 2,540 ml/L.d보다 약 53%로 높았다.

이상 결과로 산화전극과 환원전극으로 구성된 생물전기화학장치를 혐기성소화조 설치하면 유기물제거, 메탄발생량 및 메탄수율 등 소화효율은 크게 향상시킬 수가 있어 전통적인 혐기성소화기술의 단점들이 극복할 수 있는 것으로 판단하였다.

KEY WORDS: Bioelectrochemical Anaerobic Digestion 생물전기화학적 혐기성소화; Direct Interspecies Electron Transfer 직접종간전자전다; Sewage Sludge 하수 슬러지; Acidic Distillery Wastewater 산성 주정폐수; Electroactive Bacteria 전기활성미생물

Chapter 1: Introduction

1.1 Background

Anaerobic digestion process is one of the oldest technologies for treatment of organic material and thereby recovering clean energy. It is generally recognized that anaerobic digestion is a more sustainable and controllable way to treat organic matters as compared with other disposal routes such as landfill and composting (Guo et al., 2013). Anaerobic digestion has been applied to manage sewage sludge, agricultural waste, distillery wastewater, food waste and other high strength organic wastewater (Khalid et al., 2011, Mao et al., 2015). However, anaerobic digestion still has disadvantages because of the process instability, low methane content in biogas (<65%), and low organic matter degradation (<50% for sewage sludge) (Song et al., 2004, Song et al., 2016). These disadvantages are mainly caused due to the low growth rate of hydrolytic bacteria and methanogenic bacteria, which are relative slower than acidogenic bacteria (Song et al., 2004; Chen et al., 2007). Therefore, anaerobic digesters were generally operated at a low organic loading rate, or a long HRT (>20 days), and maintained at a stable temperature (35 or 55 ℃) (Song et al., 2004; Bolzonella et al., 2005; Chen et al., 2008). A lot of researchers are looking to achieve high efficiency in anaerobic digestion by studying pretreatment methods, such as, aicd/alkaline treatment, ultrasound treatment, and heat treatment, for increasing the growth rate of hydrolytic bacteria. However, the pretreatment methods only achieved limited success in increasing the efficiency of anaerobic digestion, that is not considering their high cost (Esposito et al., 2012; Mata-Alvarez et al., 2014).

Recently, bioelectrochemical technology is widely used for organic matter treatment through a high redox rate between the anode and cathode, in addition to being less sensitive to external environment, such as, influent pH, and temperature (Kumar et al., 2017). Many waste treatment systems, when coupled with bioelectrochemical technology achieved high organic matter

reduction rate and simultaneously produced clean energy, such as microbial fuel cells (MFCs), microbial electrolysis cells(MECs), microbial electrosynthesis (MES), microbial solar cell (MSC), and microbial desalination cell (MDC) (Liu et al., 2012; Song et al., 2015a; Kumar et al., 2017). Bioelectrochemical technology (BET) can be coupled with anaerobic digestion by installing anode and cathode inside an existing conventional anaerobic digester, resulting in high organic removal efficiency and methane production rate (Jiang et al., 2013; Gajaraj et al., 2017; Kumar et al., 2017). The bioelectrochemical anaerobic digestion for methane production shows several advantages compared to conventional anaerobic digestion processes, such as: (i) less thermal energy is required to maintain the temperature for the process; (ii) richermethane production and methane content in biogas; (iv) both high concentrations and diluted streams of organic waste can be used as substrate for the process; (ii) the methanogenic consortia are less sensitive to the external environment, such as temperature, influent pH etc. (Villano et al., 2011; Song et al., 2016).

In a bioelectrochemical anaerobic digester, organic matter is oxidized on the anode surface into electrons, protons and carbon dioxide. The electronsare transferred from the anode to the cathode through an external circuit via the applied voltage, and then the oxidized products are reduced on the cathode surface to form methane (Song et al., 2016; Cheng et al., 2009). Therefore, the electrode is one of the important factors in a bioelectrochemical anaerobic digester. Several studies on bioelectrochemical electrodes, including anode and cathode, have been conducted, however, most studies focused on microbial fuel cells. For bioelectrochemical anaerobic digesters, the information available on electrodes is limited. The general considerations for a bioelectrochemical anode are i) wide surface area, ii) electrochemical catalytic activity, iii) highelectric conductivity, iv) biocompatibility, v) chemical stability, vi) mechanical strength, vii) low price of materials, etc. (lee et al., 2012; Song et al., 2015b; Feng & Song, 2016a). The bioelectrochemical cathode should also have the same chemical andphysical properties of the anode, however,

high catalytic activity is required in addition for reducing carbon dioxide into methane (Kadier et al., 2015; Feng & Song, 2016b). Despite the need, studies are sparsely conducted to find suitable electrodes for a bioelectrochemical anaerobic digester.

In previous study, several papers published the effects of applied voltage on MEC in hydrogen production, and summarized that the optimal voltage for hydrogen production is approximately in the range of 0.8V - 1.2V (Kadier et al., 2014; Tartakovsky et al., 2009; Wang et al., 2009). However, there are very few studies related to bioelectrochemical methane production up until now, and most of them had used artificial wastewater as substrate in batch reactors at mesophilic temperature condition (Nam et al., 2011; Feng & Song, 2016a; van Eerten-Jansen et al., 2015). The electrode potential effects on bioelectrochemical reactions for the application of sewage sludge at low temperatures are yet to be studied. Moreover, electrochemically active bacteria (EAB) acting as a biocatalyst improves the biochemical reaction on the electrode, which has been proven to be less sensitive to temperature (Larrosa-Guerrero et al., 2010; Feng et al., 2016a). Thus, it is expected that bioelectrochemical anaerobic digestion is possibly less sensitive to temperature than conventional anaerobic digestion. However, the temperature dependence in bioelectrochemical anaerobic digestion has been sparsely investigated until now.

Summing up the above, the bioelectrochemical anaerobic digestion is certainly influenced by the parameters including electrode materials, applied voltage, temperature, as well as organic loading rate, influent pH, reactor design and some other unknown factors. The studies on the operational parameters of bioelectrochemical anaerobic digestion are urgently required for achieving high anaerobic methane production.

1.2 Objective

In this study, the optimal design and operation parameters of

bioelectrochemical anaerobic digestion is investigated. Carbon based anode and cathode were developed, and their effects on bioelectrochemical anaerobic digestion was examined in batch reactors. The influence of hydraulic retention time, temperature and applied voltage on bioelectrochemical anaerobic digestion of sewage sludge was studied. In order to explore the relationship between anaerobic planktonic bacteria and electroactive bacteria, bioelectrochemical anaerobic batch digesters were operated with different amounts of anaerobic sludge. Moreover, the distillery wastewater, which is one of the most complex types of wastewater, was used in an upflow anaerobic bioelectrochemical reactor (UABE) and compared to an upflow anaerobic sludge blanket (UASB).

1.3 Scope and content

The scope and content of this study consist of 4 parts for achieving the thesis purpose, and they are as follows:

① Electrode of bioelectrochemical anaerobic digestion

For the anode, an attempt to improve electrochemical properties of Graphite fiber fabric (GFF) was carried out by pretreating via sonication, and electrophoretic deposition (EPD), which uses multiwall carbon nanotube (MWCNT) and nickel. The GFFs were further modified to complete the anodes by forming a scaffold layer with a paste of EG and MWCNT using either coal tar pitch or epoxy as the binding agent. It was examined to determine how the anode modifications affected start-up, and performance of the bioelectrochemical anaerobic digester.

As for the cathode, three different types of cathodes were prepared by decorating MWCNT with three catalytic materials (Ni, Fe and ammonia) and then electrophoretically depositing them onto the surface of Graphite fiber fabric (GFF). Bioelectrochemical methane production from the three cathodes was examined in batch anaerobic reactors.

② Influence of HRT, temperature and applied voltage on bioelectrochemical anaerobic digestion for sewage sludge treatment

A lab scale bioelectrochemical anaerobic digester was designed and semi-continuously operated with sewage sludge. The hydraulic retention time (5, 10, 15, 20 days) and temperature (25 & 35℃) was investigated at a low applied voltage of 0.3 V between anode and cathode. The applied voltage was also varied in the range of 0.3 V - 0.7 V in the bioelectrochemical anaerobic digester. The performance of the digester including digester stability, organic matter removal, biogas production, and methane content in the biogas were monitored and compared.

③ Electron transfer pathways for methane production in bioelectrochemical anaerobic digestion

The features of bioelectrochemical anaerobic digestion was compared and discussed to conventional anaerobic digestion in terms of electron transfer pathways. The influence of planktonic bacteria in the bulk solution on electron transfer pathways was also investigated in a bioelectrochemical anaerobic batch experiment by varying the amount of anaerobic sludge (0, 100, 200, 400mL), after maturing electroactive bacteria on the electrode.

④ Performance of upflow anaerobic bioelectrochemical reactor for acidic distillery wastewater treatment

The influence of influent pH and effluent recirculation on methane production were investigated in an upflow anaerobic bioelectrochemical reactor using acidic distillery wastewater and compared to that of an upflow anaerobic sludge blanket reactor. The electron transfer pathway for methane production was investigated, and the methane production, methane content in biogas, organic matter removal, sulfate removal, and digester stability were monitored and compared.

Chapter 2: Literature Review

2.1 Anaerobic digestion

2.1.1 Fundamentals of anaerobic digestion

Anaerobic digestion process is used for the treatment of organic material to recover the clean energy over 100 years, and it has been applied to manage organic wastes such as sewage sludge, agricultural waste, food waste and other high strength organic wastewater (Khalid et al., 2011, Mao et al., 2015). The anaerobic digestion is carried through by the microorganisms living in oxygen-free condition, and the anaerobic conversion process consists of biochemical reactions balanced in hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 2.1) (Shin & Song, 1995; De la Rubia et al., 2002; Song et al., 2004). During the hydrolysis, the insoluble organic polymers contained in wastewater, such as Lipids, carbohydrates and proteins are first broken down into soluble monomers. In the acidogensis, the monomeric materials, including fatty acids, amino acids and sugars, are fermented into small organic molecules such as ammonia, organic acids, hydrogen and carbon dioxide by the acidogenic bacteria. Then, the acetogenic bacteria convert the organic acids into acetic acid, formate, hydrogen and carbon dioxide by the generated electrons from a substrate. Finally, the hydrogen and carbon dioxide are converted to methane by the hydrogenotrophic methanogens, and the small organic molecules are converted into methane and carbon dioxide by acetoclastic methanogens (Lyberatos & Skiadas, 1999; Gutierrez et al., 2009; Mao et al., 2015). In the anaerobic digestion, the different microorganisms involved in the reaction at each stage, but the acidogenic bacteria and methanogenic bacteria are dominant (Table 2.1). The anaerobic conversion steps are catalyzed by different species of anaerobic microbial groups, which have different metabolic pathways and environmental conditions.

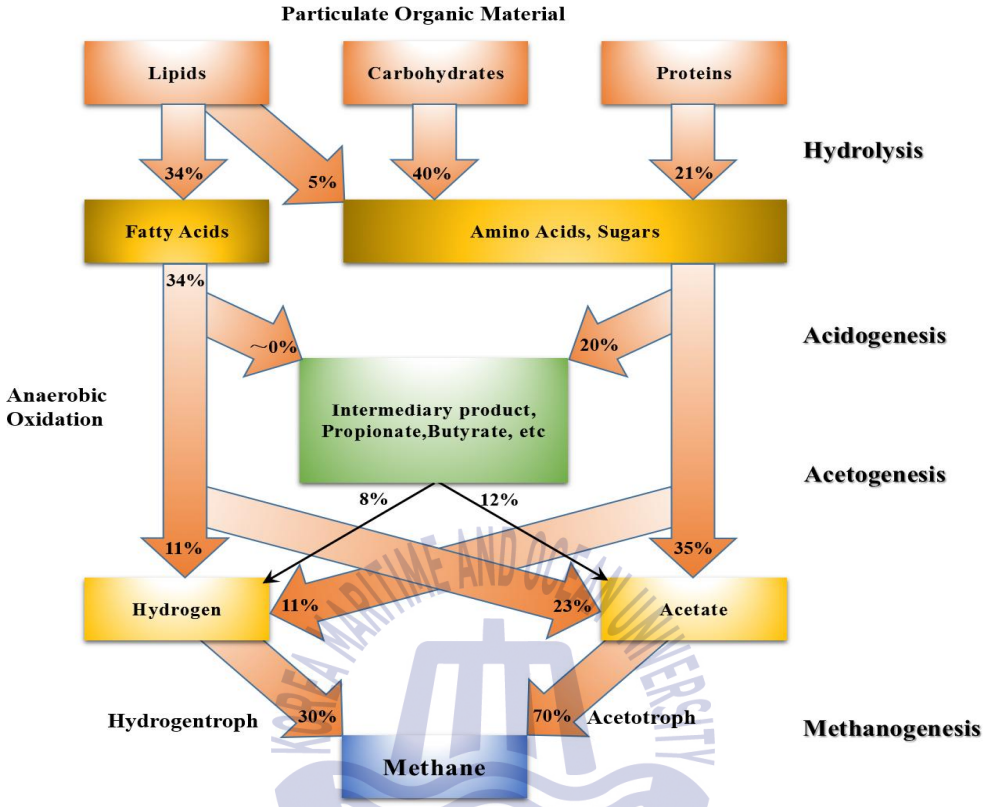


Fig. 2.1. Anaerobic degradation pathway of polymeric biomass to methane (Henze, et al., 1983).

Table 2.1 Substrates and products for the main anaerobic microbes

Anaerobic bacteria	Substrate	Products
Methanogens	Acetic acid, methanol, CO_2, and formic acid	Methane
Hydrolysis bacteria	Complex organic matter	Monomer
Denitrifying bacteria	Oxidized nitrogen	N_2, NH_3, N_2O
Sulfate reducing acteria	Sulfate	H_2S
Acetogens	Short chain fatty acids	Acetic acid
Homoacetogens	formic acid, propionic acid compounds	Acetic acid

① Hydrolysis

During the first stage of hydrolysis, the extracellular enzymes convert particulate organic matters (lipid, carbohydrate, protein) into liquefied monomers and polymers such as fatty acids, amino acids, sugars and other carbohydrates, are readily available to other bacteria (Fig. 2.2) (Guerrero et al., 1999; Luo et al., 2012b). The stabilization of the organic matter does not occur during the hydrolysis, but it is converted into the form that easily used by acetogenic bacteria and the reaction is relative slow. Thus, energy from the hydrolysis cannot be obtained, but it is an essential step for the next step of the anaerobic reaction. Equation 2.1 shows the glycogen is broken down into a simple sugar in the hydrolysis reaction (Ostrem & Themelis, 2004; Zhang et al., 2009a; Luo et al., 2012b).

Equation 2.1: $C_6H_{10}O_5 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2$

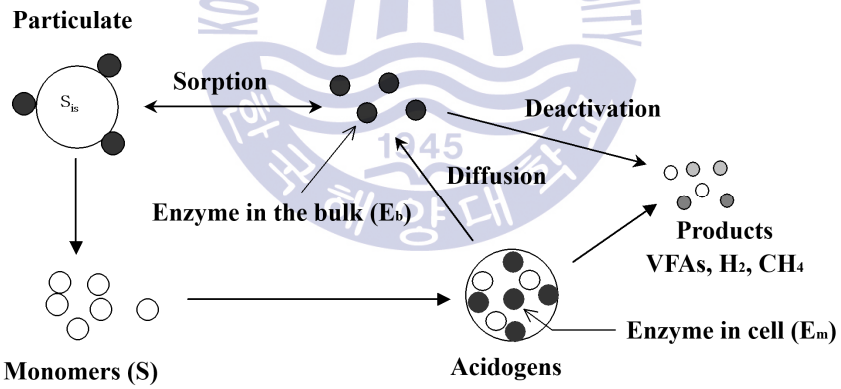


Fig. 2.2. Schematic diagram of hydrolysis model.

② Acidogenesis

In the second stage, the sugars, amino acids, long chain fatty acid generated from hydrolysis are converted into carbon dioxide, hydrogen, ammonia, and organic acids by the acidogenic bacteria such as Syntrophomonas wolfei and

Syntrobacter wolinii.(Guerrero et al., 1999) The principal acidogenesis stage products are ethanol, methanol, formic acid, acetic acid, lactic acid, butyric acid, propionic and others. From these products, the carbon dioxide, acetic acid and hydrogen will skip the third stage (acetogenesis), and be used directly by the methanogenic bacteria in the methanogenesis stage (Fig. 2.1). The glucose converted to acetic acid, propionate and ethanol in the typical acidogenesis showing in the equation 2.2-2.4 (Guerrero et al., 1999; Ostrem & Themelis, 2004; Strauber et al., 2012; Zheng et al., 2015)

Equation 2.2: $C_6H_{12}O_6 \rightarrow 3CH_3COOH$

Equation 2.3: $C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$

Equation 2.4: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$

③ Acetogenesis

In the acetogenesis stage, the residual acidogenesis products, such as alcohol butyric acid and propionic acid are converted into acetic acid, carbon dioxide and hydrogen by acetogenic bacteria (Fig. 2.1). However, the hydrogen only occurs when the partial pressure is low to allow the conversion of all the acids thermodynamically. The hydrogen scavenging bacteria carry out a lower partial pressure, thus the hydrogen concentration in the anaerobic digestion is an important indicator (Mata-Alvarez, 2003; Cazier et al., 2015). Equation 2.5-2.7 represents the conversion of propionate, glucose and ethanol to acetate during the third stage of anaerobic fermentation (Ostrem & Themelis, 2004; Strauber et al., 2012; Zheng et al., 2015).

Equation 2.5: $CH3CH_2COO- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$

Equation 2.6: $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$

Equation 2.7: $CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$

④ **Methanogenesis**

In the final stage of methanogenesis, and the small organic molecules such as an acetic acid are directly converted into methane and carbon dioxide by acetoclastic methanogenic bacteria (Equation 2.8), and hydrogen and carbon dioxide are also converted into methane by the hydrogenotrophic methanogenic bacteria (Equation 2.9) (Villano et al., 2010). It is reported that 70% of methane are produced from acetoclastic methanogenic bacteria, but only 30% of hydrogenotrophic methanogenic bacteria (Smith and Mah, 1966; Balch et al., 1979). The bacteria responsible for this conversion are called methanogens and are strict anaerobes (Ostrem & Themelis, 2004; Cuzin et al., 2001; Karakashey et al., 2005). The activity of methanogenic bacteria is greatly inhibited by oxygen and it is very sensitive to environmental factors such as temperature, organic load rate, organic matter components toxic substances. Thus, the methanogenic bacteria is easily got affected from the external environment. Generally, the maximum growth rate of methanogens occurs at pH 6-8, and the maximum activities are presented at the temperature of 30-40 ℃ and of 55℃ (Song et al., 2004; Bolzonella et al., 2005).

Equation 2.8: $CH_3COOH \rightarrow CH_4 + CO_2$

Equation 2.9: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

2.1.2 Factors affecting anaerobic digestion

① **Components of organic matter**

In anaerobic digestion, the influent organic wastes are an important substrate for anaerobic microorganisms. Generally, the organic matter consists of substances that are easily degradable and difficult to decompose, and not all of substances can be decomposed in anaerobic digestion (Chen et al., 2008, Cuzin et al. 2001). The degradable material is biologically degradable by the anaerobic microorganism, resulting in high treatment efficiency and a large

amount of biogas production. However, some un-degradable substances are difficult to decompose under anaerobic conditions and accumulate in the digester to prevent decomposition. Therefore, the anaerobic digester should be operated on the basis of the amount of biologically degradable organic matter according to the characteristics of the influent wastes (Chen et al., 2008. Karakashev et al., 2005)

② Organic loading rate

The organic loading rate is the influent amount of biologically degradable organic matter per unit volume of the anaerobic digester (Song et al., 2016). The low organic loading rate for anaerobic digestion results in a small amount of biogas production. On the other hand, when the organic loading rate is high, the volatile fatty acid can be overproduced, and the accumulation of volatile fatty acid resulting in a decreased pH and an unstable state (Zheng et al., 2015; Strauber et al., 2012; Cazier et al., 2015). The optimal organic load rate of the anaerobic digester can be adjusted through the operating factors (Mata-Alvarez et al., 2003; Guerrero et al., 1999).

③ HRT

The HRT of an anaerobic digester is determined by the volume of organic matter input per day, and it is influenced by seasonal factors (Luo et al., 2012b). In the single phase anaerobic digestion process, the HRT is often designed to be in a range of 20-30 days (Song et al., 2004). In the case of the two-phase anaerobic digestion process, the first anaerobic digestion tank should be over 20 days and the second anaerobic digestion tank should be over 10 days (Song et al., 2004; Lyberatos & Skiadas, 1999; Chen et al., 2008). However, when the anaerobic digester is operated at ambient temperature without the heating system, the suitable HRT will be 60 to 90 days (Ostrem & Themelis, 2004; Cuzin et al., 2001).

④ pH

PH is one of the most important environmental factors for the operation and management of anaerobic digester (Song et al., 2004; Luo et al., 2012b). In general, the optimal pH of the anaerobic digestion is between 6.5 and 8. The accumulation of volatile fatty acids can reduce the ability of methanogenic microorganisms to respond to environmental changes such as the characteristics of influent wastes, changes in temperature, and toxic substances, thus lowering the pH of the anaerobic digester (Karakashev et al. 2005). In this case, even the acetogenic bacteria are active at low pH for increasing the growth rate of acetogenesis, but the organic acid products cannot be covered to methane and accumulated for further decreasing the pH. The alkalinity with buffering properties such as bicarbonates helps maintain the pH by neutralizing the volatile fatty acids (Guerrero et al., 1999). However, if the acid accumulates continuously, the buffering capacity decreases while being used to neutralize the acid. the buffering capacity disappears while being used to neutralise the acid and decreases the pH again (Shin & Song, 1995).

⑤ Temperature

The methanogenic bacteria, therefore, grows slowly, and methanogenesis is susceptible to changes in environmental conditions such as pH, temperature and toxic substances (Zhang et al., 2009a; Yuan et al., 2016), leading to the imbalance between the anaerobic conversion steps. The temperature condition in the anaerobic digestion process is an important factor influencing the growth of methanogenic bacteria (Song et al., 2004; Varel et al., 1980; Masse et al., 2015). The anaerobic digestion process is commonly classified into psychrophilic- (<20 ℃), mesophilic- (25-45 ℃) and thermophilic digestion (>45 ℃), according to operation temperature (Song et al., 2004; Zhang et al., 2009a; Masse et al., 2015; Connaughton et al., 2006). The anaerobic degradation rate of organic matter is generally higher at higher

temperature ranges (Song et al., 2004; Zhang et al., 2009a; Zhang et al., 2006). However, the anaerobic digestion process in higher temperature ranges is relatively unstable and requires additional energy for heating (Song et al., 2004; Mao et al., 2015; Varel et al., 1980; Guerrero et al., 1999; Zupancic et al., 2003; Labatutu et al., 2014). Therefore, mesophilic anaerobic digestion (35 ℃) has advantages in anaerobic process stability, high methane yield and less heating energy requirements for the digester compared to thermophilic digestion (Labatut et al., 2014). Commonly, mesophilic digestion is recognised as a reasonable process in spite of its moderate performance in organic matter removal and methane production rate. However, it is pointed out that a large amount of heating energy is required for the mesophilic digestion, and the stability of digester operation is low (Song et al., 2004; Song et al, 2016).

⑥ VFA

Many researchers have noted that volatile fatty acid (VFA) concentrations are one of the most important factors in anaerobic digestion (Komemoto et al, 2009; Kondaveeti & Min, 2015). The concentration and composition of volatile fatty acids are the best indicators for anaerobic digestion. When the concentration of volatile fatty acids exceeds the allowable concentration, the activity of the methanogenic bacteria can be inhibited and the production speed of VFA is faster than the decomposition rate. Then, as mentioned above, the accumulation of volatile fatty acids reduces pH and prevents anaerobic digestion. The accumulation of volatile fatty acids is a symptom of an unstable state of the biological system because it is the result of the difference in the rate of reaction steps (Lyberatos & Skadas, 1999; Song et al., 2004).

⑦ Toxic substances

Toxic substances including ammonia, salinity, and heavy metals affects the

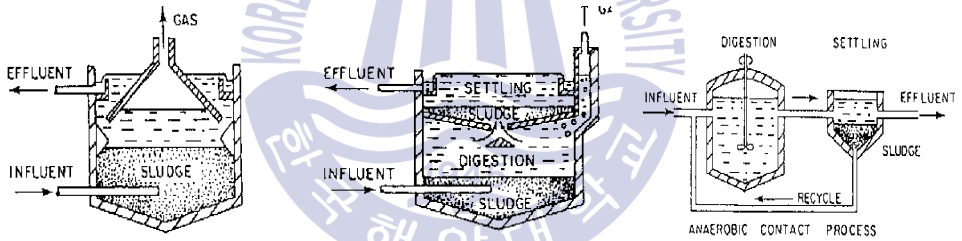
performance of anaerobic digestion. Generally, the soluble heavy metals are not highly hazardous because they can be formed into precipitate metal sulfates in the presence of sulfur. However, the ammonia ions have been reported to act as inhibitors of anaerobic digestion and toxic to methanogenic bacteria (Angelidaki et al., 1993). The amount of protein in the influent waste can be estimated from the C/N ratio. When the concentration of salt is high, most microorganisms are dehydrated by the osmotic pressure and the growth is inhibited. In particular, sodium ion has a strong inhibitory effect on anaerobic digestion. In addition, anaerobic microorganisms have been reported to suffer from heavy metals such as chromium, cadmium, copper, lead, zinc, nickel and cobalt etc. (Lyberatos & Skiadas, 1999; Mata-Alvarez et al., 2003).

2.1.3 Digester types and application of anaerobic digestion

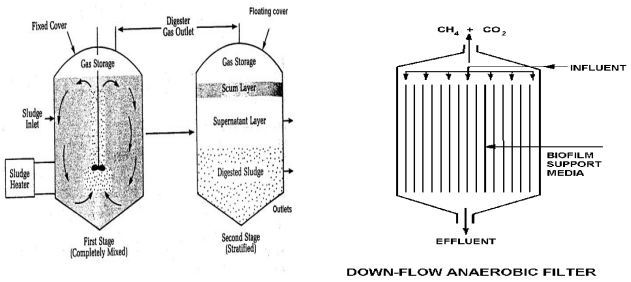
Anaerobic digestion is a green technology, which is capable of recovering energy such as methane gas while stabilizing organic matter, has been widely used for treating organic matters. The basic form of the anaerobic digester is mainly composed of complete mixed type, two-stage type and tube flow type shown in Fig. 2.3 (a, b, c). However, these conventional anaerobic digester have disadvantages such as low organic matter removal rate, high carbon dioxide content in biogas, relative unstable process due to the slow growth rate of the methanogens and sensitivity to the environment. Many researchers were trying to develope the high-rate anaerobic digester that overcome these shortcomings and improve digestion efficiency, such as upflow anaerobic sludge blanket (UASB), anaerobic clarigester, anaerobic contact process, tow stage sludge digester and anerobic filter (Fig. 2.3d-h). In particular, the UASB is designed to prevent the large amount of solids flowing out from the reactor, which can maintain a high density of microorganisms in the system. In South Korea, anaerobic digestion is widely using in various fields for organic matter treatment and biogas production, such as sewage sludge, biowaste and agriculture. In the last three decades, the number of anaerobic

wastewater treatment plants have gradually increased (Fig. 2.4). The total number of anaerobic plants in South Korea was 71 in 2014, including 45 sewage sludge plants, 20 biowaste plants and 6 agriculture waste plants (Table 2.2). A total of 71 anaerobic plants are now in operation to produce as much energy as 2,603 GWh per year, which expresses the biogas energy potential. The biogas energy produced from sewage sludge is 70.8%, higher than biowaste (28.9%) and agriculture waste (0.3%). Biowaste mainly consists of food waste, food waste leachate, and digestible co-substrates (Kang, 2015).

(a) Mixed type digester (b) Two stage digester (c) Plug flow digester



(d) Upflow anaerobic sludge blanket (UASB) (e) Anaerobic clarigester (f) Anaerobic contact process



(g) Two stage sludge digester (h) Anaerobic filter

Fig. 2.3. Types of anaerobic digester.

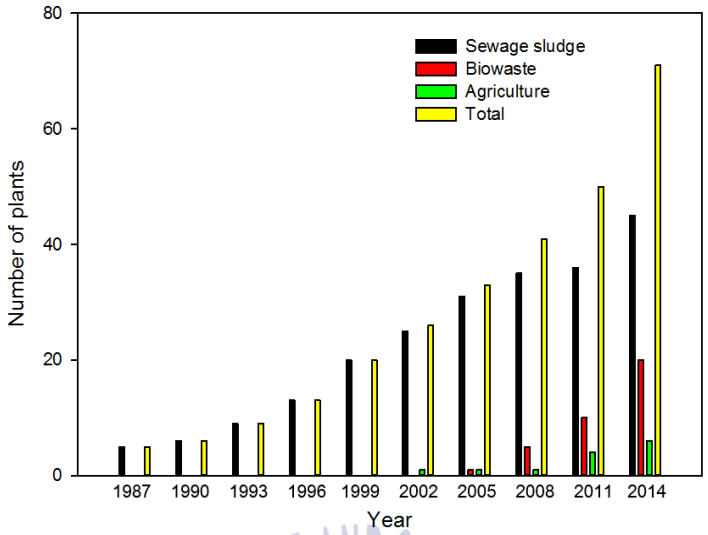


Fig. 2.4. Growth trends of anaerobic digestion in South Korea

Table 2.2 Application of anaerobic digestion in South Korea in 2014

Plant type	Number of plants	Biogas production* (GWh/Year)
Sewage sludge	45	1,045
Biowaste	20	427
Agriculture	6	3
Total	71	1,475

*Expressing as biogas energy content from different anaerobic plant

2.1.4 Merits of anaerobic digestion

The anaerobic digestion process can be used to treat high concentration of organic wastewater and waste compared to aerobic processes, and it has advantages such as low operating costs and utilization of biogas as a byproduct. The biogas generated from the anaerobic digester is usually used

as a raw material for power generation or for producing thermal energy through a boiler. Recently, anaerobic digestion technology has been used for the treatment of various industrial organic wastewater, and is recognized as a more environmentally-friendly and economical technology (Ostrem & Themelis, 2004; Zheng et al., 2015).

The anaerobic process can efficiently treat the concentrated organic wastewater which is difficult to treat by aerobic process. The anerobic digestion produces relatively low sludge and the methane gas generated from anaerobic process can be utilized as alternative fuel energy. The anaerobic digestion also has an advantage in which it does not require the aeration device used in the aerobic treatment process. In addition, the anaerobic digestion process is relatively easy to dehydrate and dry the residual sludge, thus increase the mortality rate of parasites and epidemics (Song et al., 2004; Luo et al., 2012b).

However, the anaerobic digestion also has lots of disadvantage due to the slow growth rate of methanogen and its sensitivity to environmental changes (Stauber et al., 2012; Cazier et al., 2015). In the conventional anerobic digester, the HRT required is more than 20 days, the removal rate of organic matter is low, and the operating conditions are disadvantageous. Also, the volume of anaerobic digester is relatively large because of the slow growth rate of hydrolysis and methanogenesis. The bacteria, which is a major component of sewage sludge, has a thick cell wall and a dense membrane composed of cellulose, lignin, and silica, which have low biodegradability. Thus, the VS removal efficiency is not high in the conventional anaerobic digestion for sewage sludge. The odor can be occurred when the BOD is high in the anaerobic digester, and the investment cost is large due to the necessity of the supplementary facilities (Song et al., 2004; Chen et al., 2008; Song et al., 2016).

2.2 Bioelectrochemistry

2.2.1 Definition of bioelectrochemistry

Bioelectrochemistry is a branch of electrochemistry and biophysical chemistry concerned with electrophysiological topics like cell electron-proton transport, cell membrane potentials and electrode reactions of redox enzymes (Song et al., 2015a; Kumar et al., 2017). Bioelectrochemical systems are engineered systems in which the electronic transfer chain associated with microbial respiration is short-circuited. Electrons that naturally flow from the substrate towards oxygen or another electron acceptor are collected at an electrode, on which the microorganisms form a biofilm (Kumar et al., 2017). Like in all fuel cells, the anodic reaction is coupled to an electron consuming reaction at a cathode. If the oxidation of organic matter at the anode is coupled to the reduction of oxygen at the cathode, the positive cell potential and the flow of electrons results in electricity production. Alternatively, oxidation of organics is coupled to hydrogen production. The valorization of organic wastes by electricity or hydrogen production is the usually foreseen application of bioelectrochemical systems (Cheng et al., 2007; Deng et al., 2010; Ghasemi et al, 2013).

2.2.2 Applications of bioelectrochemistry

Recently, the bioelectrochemical system has been widely investigated for intermediate chemical production and useful resource recovery. Certain amounts of resource in organic waste can be used as reducing power for the electric production, as well as the useful energy recovered from organic waste by using a novel inter-disciplinary biotechnology, and treating the organic waste simultaneously (Kim et al., 2015a). Recent applications of bioelectrochemical system for useful resource recovery, such as the microbial electrolysis cell (MEC), microbial fuel cell (MFC), microbial desalination cell (MDC), microbial solar cell (MSC) and microbial electrosynthesis (MES) as shown in Fig. 2.5 (Kumar et al., 2017).

In MEC, the electroactive bacteria are capable of transforming waste substrate into electrical energy by applying a small electric energy, and then produce other form of chemical energy via oxidation-reduction reactions on the electrodes, such as hydrogen and methane (Liang et al., 2011; Liu et al., 2012). Whereas in MFC, the electron produced by the electroactive bacteria attaching on the anode and then transfered to cathode by an external electric circuit, which forms the current for electricity generation (Lee et al., 2012; Song et al., 2015a). MDC was introduced to involve concurrent desalination with hydrogen retrieval (Kumaret al., 2017). The MSC uses photoautotrophs or photosynthetic bacteria to perform electrode-driven reactions by absorbing the light from solar, then consumed by the electroactive bacteria. These electroactive reactions comprise formation of electric current and byproducts, such as ethanol, methane and hydrogen (Kim et al., 2015a; Kumar et al., 2017). The MES can be also used for the biochemical products and other fuels such as biogas and bioalcohols by the reaction on the cathode (Van Eerten-Jansen et al., 2015; Cheng et al., 2009). Therefore, the bioelectrochemical system can be considered a feasible approach for treating organic matter and producing energy fuels, simultaneously. (Kumar et al., 2017).

2.2.3 Trends in bioelectrochemistry

During the past 20 years, many studies have concentrated on improving the performance of bioelectrochemical system for waste treatment and energy production. However many technical, scientific and economic challenges still be addressed for achieving the high performance of bioelectrochemical system to make it commercially available (Rabaey et al., 2010; Kumar et al., 2017). The effects of factors such as electrode materials, reactor design and configuration and operation conditions on the performance of bioelectrochemical system have been mostly studied in lab scale reactors, but not be addressed for achieving its full potential at commercial scale yet (Zhou et al., 2011; Song et al., 2015ab; Kim et al., 2015a). It is often under

controlled and ideal conditions for the experiments of bioelectrochemical system in the laboratory, and it should be proved that works reliably at a larger scale and for sustained periods of time in the nature conditions, but is essential if commercialization of the technology is to be realized. (Clauwaert et al., 2008; Nam, et al., 2011). For the commercialization of bioelectrochemical technology, low-cost materials for the electrode, digester design are very important factors. Constructing the high surface area, cheap, and conductivity electrode and improvements in designs of bioelectrochemical system will significantly improve the practical utilization in the commercialization for energy recovery from the organic waste (Kim et al., 2015a; Kumar et al., 2017). As a result, further studies are required for a better understanding of bioelectrochemical technology and to improve its use in commercial applications.

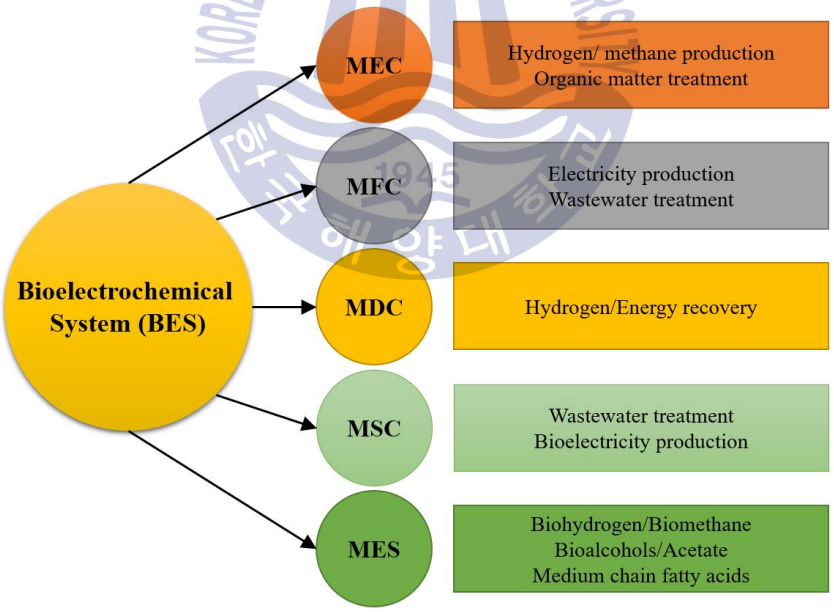


Fig. 2.5. Schematic overview of various types of bioelectrochemical system.

2.3 Bioelectrochemical anaerobic digestion

2.3.1 Working principle of bioelectrochemical anaerobic digestion

Bioelectrochemical technology (BET) can be coupled with anaerobic digestion by using the theory of microbial electrolysis cell. The result of which is installing anode and cathode inside an existing conventional anaerobic digester, and maintaining a small potential difference between the anode and cathode (Fig. 2.6) (Song et al., 2016). Generally, the majority of methane production was attributed to electrode in a bioelectrochemical anaerobic digester. Organic matter is oxidized on the anode surface into electrons, protons and carbon dioxide. The electrons are transferred from the anode to the cathode through an external circuit via the applied voltage, and then the oxidized products are reduced on the cathode surface to form methane. The reactions on the surface of anode and cathode are described by equation 2.10 and equation 2.11, respectively (Song et al., 2016; Cheng et al., 2009).

Equation 2.10: $CH_3COOH+2H_2O \rightarrow 2CO_2+8H^++8e^-$, E_{pa}=-0.486V (vs. Ag/AgCl)

Equation 2.11: $CO_2+8H^++8e^- \rightarrow CH_4+2H_2O$, E_{pc}=-0.445V (vs. Ag/AgCl)

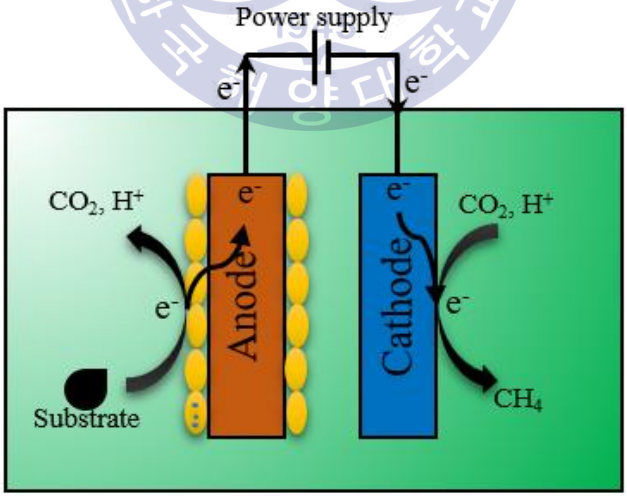


Fig. 2.6. Schematics of bioelectrochemical anaerobic digestion.

In recent studies, the methane production at the electrode surface of a

bioelectrochemical anaerobic digester is reported to be less than 20% (Zhao et al., 2015; Shen et al., 2016). It is also mainly attributed to the enhanced direct interspecies electron transfer (DIET) by the enrichment of electroactive bacteria (Kato, 2015). When a redox compound is present in an anaerobic digester, it was observed that the electroactive bacteria reduce the redox compound to transfer the electron, and then the methanogenic bacteria use the electron from the compound to produce methane (Lovley, 2011; Marsili et al., 2008; Richter & Gescher, 2014; Shen et al., 2016; Shrestha et al., 2014). It is known that when the methanogenic bacteria are in close proximity to the electroactive bacteria via either the anode and cathode in an anaerobic reactor coupled with bioelectrochemical devices, or through a conductive material in the anaerobic reactor, the methanogenic bacteria produce methane from the reduction of carbon dioxide using the electrons transferred directly from electroactive bacteria (Dube & Guiot, 2015; Rotaru et al., 2014a; Shen et al., 2016; Shrestha et al., 2014). These types of electron transfer pathways for methane production are referred to as direct interspecies electron transfer (DIET). However, the electron transfer pathway for methane production in the bioelectrochemical anaerobic digester is sparsely studied.

2.3.2 Factors affecting bioelectrochemical anaerobic digestion

① Electrode

The bioelectrochemical electrodes, including anode and cathode, have been studied earlier, however, these studies were primarily focused on microbial fuel cells (Song et al., 2015ab; Nan et al., 2011). The available information on bioelectrochemical electrodes is still limited. The general considerations for bioelectrochemical anode are as follows: i) high conductivity, ii) affinity for microorganisms growing, (iii) a porous material having a large specific surface area for microorganisms attaching, (iv) chemical and biological stability and durability, (v) the shape of the electrode should be easily manufactured, (vi) inexpensive materials, (vii) no clogging for the overgrowth

of microorganisms, (viii) easy scale-up for bioelectrochemical anaerobic digestion (Liang et al. 2011; Song et al., 2015a). The bioelectronchemical cathode should also have similar characteristics with its electrode equivalent. In theory, the bioelectrochemical cathode acts as an electron acceptor, as it uses the electrons transferred from anode to reduce carbon dioxide to methane. For better efficiency, the catalyst of the cathode plays a vital role in accelerating the reaction rate of methane production from carbon dioxide. Therefore, the electrochemical properties of bioelectrochemical cathode plays an important role in determining the reaction rate. However, available information on the cathode materials is also not adequate. In order to transfer the electrons to the surface of bioelectrochemical cathode smoothly, a highly conductive material is used. The bioelectrochemical cathode should have a wide specific surface area so that the reduction reaction proceeds efficiently. A suitable catalyst on the cathode can increase the efficiency of methane production by reducing carbon dixoide.

Table 2.3 Status of bioelectrochemical anaerobic digestion according to few representative studies

Substrate	Volume	Electrode materials	Voltage	Methane Production	Reference
Activated sludge	0.8 L	Reticulated vitreous carbon	0.3~0.6v	1.65 ml/L	Gajaraj et al., 2017
Artificial wastewater	0.49 L	Carbon-felt	Cathode potential: -0.85-1.15 V (vs. Ag/AgCl)	0.27 L.d	Jiang et al., 2013
Synthetic wastewater	1 L	Graphite	1.0-1.5 V	1.2 L/L.d	Li et al., 2016
Artificial wastewater	0.3 L	Ti/Ru alloy mesh plate	1.4~1.8V	0.43-0.53 L/L	Guo, et al., 2013
Sewage sludge	4.0 L	Carbon fiber fabric	Cathode potetnial: 0.8 V (vs. Ag/AgCl)	2.35 L/L.d	Sasaki et al., 2013
F-T wastewater	4.8 L	Graphite felt (GF)	1.5 V	2.31 L/L.d	Wang et al., 2017
Glucose & acetate	1 L	Graphite	0.5-1.0 V	0.94-0.99 L/L.d	Zhao et al., 2014

Until now, most carbon based materials generally meet the requirements of

the bioelectrochemical anode and cathode. Materials such as carbon paper, carbon plate, carbon cloth, graphite rod graphite granule, reticulated vitrified carbon, and multiwall carbon nano tube have been widely used as bioelectrochemical electrodes (Table 2.3) (Song et al., 2015ab; Feng & Song, 2016a,b). However, a more efficienct and durable bioelectrochemical electrode material is still required to achieve high-rate bioelectrochemical methane production.

② Applied voltage

In a bioelectrochemical anaerobic digester, the potential difference between anode and cathode is one of the most important factors for efficient operation. Organic matter is oxidized by electroactive bacteria, which adhering onto the surface of anode, and produce protons, carbon dioxide, and electrons. The electrons are transferred to the cathode, where carbon dioxide, and protons are reduced into methane by applying a small voltage with a DC power supply (Liang et al., 2011; Liu et al., 2012). Therefore, the potential difference between anode and cathode is the driving force for the electrons, which can be affected by applied voltage, internal resistance of electrode, and other external conditions (Rader & Logan, 2010; Nam et al., 2011). The bioelectrochemical reaction does not occur when the potential difference is too low (<0.2 V), but the electrolysis of water occurs if the potential difference is too large (1.48 V, theoretical value: 1.23 V) (Logan, 2008). Theoretically, anode potential should be more positive than Epa (-486 mV vs. Ag/AgCl) and cathode potential should be more negative than Epc (-445 mV vs. Ag/AgCl) (Hamelers et al., 2010). Accroding to a previous study (Wang et al., 2009), maximum amount of hydrogen gas was generated when applied voltage was in the range of 0.5- 0.9 V, but methane gas was generated when applied voltage was in the range of 0.3-0.6 V in a MEC according to a previous study. However, the applied voltage for bioelectrochemical anaerobic digestion is still sparsely studied and remains unclear.

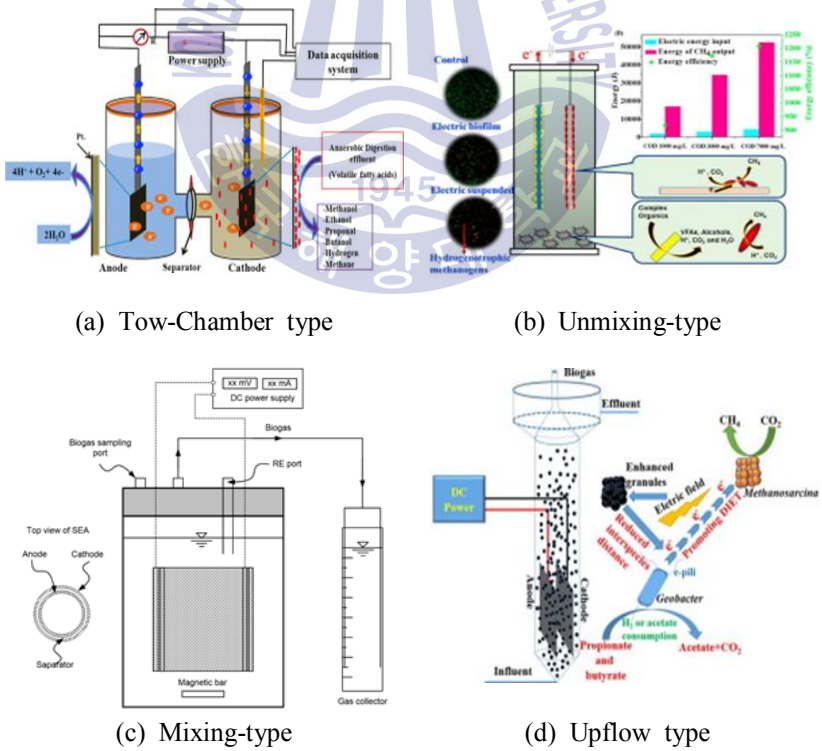
③ Others

Recently, it was proven that bioelectrochemical systems, such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), are less sensitive to external environment conditions, such as influent pH and temperature (Larrosa-Guerrero et al., 2010; Heidrich et al., 2014). In a previous study, the biogas production rate increased by 30% at a pH of 5.8 than that at 7.0 pH in a bioelectrochemical reactor (Hu et al., 2008). The methane yield in a bioelectrochemical anaerobic digester at 10℃ was 5.3 - 6.6 times higher than a control digester (without applied voltage and electrodes) operated at 10 ℃, and equivalent to the yield of a control digester operated at mesophilic condition (35 ℃) (Liu, et al., 2016). However, it is difficult to simply define that a bioelectrochemical anaerobic digester is not affected by the external environment. In theory, keeping in tune with a conventional anerobic digester, the performance of a bioelectrochemical anerobic digester is affected by influent characteristics, such as organic matter, organic loading rate, HRT, pH, temperature etc. Some researchers also published that the performance of bioelectrochemical reactors were slightly reduced by decreasing pH and temperature, but still higher than the control reactor at the same operation condition (Yuan et al., 2011; Feng et al., 2016a,b). It implies that the electroactive bacteria adhering onto the surface of electrode can be affected by external environment, but not as much as a conventional anaerobic digester.

2.3.3 Status of bioelectrochemical anaerobic digestion

Bioelectrochemical anaerobic digestion is a new and promising approach for methane production from wastewater, organic matter and other renewable resources (Kadier et al., 2014). The bioelectrochemical anaerobic digester is easily constructed by installing anode and cathode inside an existing conventional anaerobic digester, and applying a little electric energy (Song et al., 2016). The process performance in methane production and organic removal is considerably enhanced, especially the methane content in biogas is

in the range of 70% - 90% (Xafenias & Mapelli, 2014; Chen et al., 2015; Song et al., 2016; Zhao et al., 2016), which is much higher than a conventional anaerobic digester, increasing the possibility of direct application as an energy resource. However, until now, bioelectrochemical anaerobic digestion was mostly studied in small scale batch reactor using synthetic wastewater at mesophilic condition. (Wang et al., 2009; Cheng et al, 2009; Sasaki et al., 2011; Gajaraj et al., 2017). Various types of bioelectrochemical anaerobic digester like two-chambers, unmixing-type, mixing-type and upflow type shown in Fig 2.7, were used (kondaveeti & min, 2015, Li et al., 2016; Feng & Song, 2016a; Wang et al., 2017), but and it has been sparsely studied as a continuous system with complex substrates, such as sewage sludge and distillery wastewater. Therefore, bioelectrochemical anaerobic digestion requires more studies on the detailed process for the treatment of complex organic matters.



(a) Tow-Chamber type (b) Unmixing-type

(c) Mixing-type (d) Upflow type

Fig. 2.7. Reactor types of bioelectrochemical anaerobic digestion.

Chapter 3: Electrode of Bioelectrochemical Anaerobic Digestion

3.1 Introduction

Recently, anaerobic digestion coupled with bioelectrochemical technology has significantly improved the performance in methane production and organic matter stabilization (Guo et al. 2013; Tian et al., 2014; Song et al., 2016). The coupled bioelectrochemical anaerobic digestion system can be easily constructed by installing an anode and cathode inside the anaerobic digester, and maintaining a small potential difference between the electrodes (Rader & Logan, 2010; Ran et al. 2014). In the bioelectrochemical anaerobic digester, some groups of electroactive bacteria (EAB) growing on the anode surface oxidize the anaerobic intermediates such as volatile fatty acids into electrons, protons, and carbon dioxide. The electron is transferred to the anode and moved to the cathode through a conductive external circuit. Meanwhile, the other group of EABs on the cathode surface fulfils the bioelectrochemical methane production by reducing carbon dioxide to methane (Guo et al., 2013; Rabaey et al., 2010; Villano 2011). The rates of oxidizing the anaerobic intermediates and transferring the electron to the anode are governed by the activity of the EAB biomass growing on the anode surface (Sun et al., 2015; Ullery et al., 2015). The activity of EAB on the anode is closely associated with the species of EAB and their growth, which are affected by the physicochemical properties of the anode. Therefore, the anode properties are very important for a well-established bioelectrochemical anaerobic digester. Until now, although several studies on the bioelectrochemical anode have been performed, most of these have focused on microbial fuel cells. In bioelectrochemical anaerobic digester, the available information on the bioelectrochemical anode is limited yet. The general considerations for the bioelectrochemical anode are i) biocompatibility, ii) wide surface area, iii) high electrical conductivity, iv) chemical stability, v) mechanical strength, vi) electrochemical catalytic activity, vii) the price of anode material, etc (Liang

et al., 2011; Sun et al., 2011; Wu et al., 2014a). It is well known that carbon based electrode materials are generally biocompatible, conductive, and chemically stable. To date, several carbon based materials, such as carbon fiber, carbon felt, carbon nanotube, and graphene, have been examined as the anode for microbial fuel cells (Zhang et al., 2013; Yu et al., 2015; Villano et al., 2013; Zhang et al., 2012; Tang et al., 2015). Among these materials, a recommended electrode material is graphite fiber fabric (GFF) due to its mechanical strength, flexibility and wide surface area. However, some physical and electrochemical properties of GFF such as hydrophilicity, smooth surface for bacterial attachment, catalytic activity and electrical conductivity are not enough to use for an anode material in bioelectrochemical system. Several physico-chemical treatments using acid, alkali, surfactant, and heat have been examined to alter the properties of the carbon surface (Liu et al., 2014; Luo et al., 2015). Some materials such as multiwall carbon nanotube (MWCNT), exfoliated graphite (EG), conductive polymers, and some metal catalysts were quite effective to improve the properties of the GFF (Yu et al., 2015; Lee et al., 2012; Rahimnejad et al., 2015). The scaffold layer formation on the surface with these modification materials is a common approach to modify the surface properties. The scaffold layer could be formed by screen-printing of a paste of the modification materials on the surface. The paste is prepared by mixing of the modification materials and a binding agent for the adhesion and the interconnection. Several polymers, such as Nafion solution, epoxy, polyvinyl alcohol, and polytetrafluroethylene, have been examined as the binding agent (Ghasemi et al., 2013; Song et al., 2014; Zhang et al., 2007). However, these polymers as the binding agent have still some problems in low conductivity and biocompatibility, weak binding force, and high price, etc.

On the other hand, the bioelectrochemical reaction on the cathode is catalyzed by methanogenic species of EAB growing on the cathode. The methanogenic species and their biomass on the cathode surface during the enrichment of EAB are affected by the physical and electrochemical properties of the

electrode, such as roughness, surface area, electric conductivity, and electric potential (Bieefinger et al., 2009; Nan et al., 2011; Jiang et al., 2013; Xafenias & Mapelli, 2014; Song et al., 2015a; Feng & Song, 2016a). In particular, the electrochemical properties of the cathode materials are an important factor that influences the catalytic activity of EAB on the cathode. However, available information on the cathode materials is still not adequate. It is well known that several carbon based materials, such as carbon cloth, carbon felt and carbon fiber, are biocompatible, durable in chemical solution and reasonable in price. Among them, some porous carbon materials in the forms of felt and fabric have wide surface area available for bacterial growth, and recommended as good raw materials for cathode (Tsai et al. 2009; Deng et al., 2010; Wei et al., 2011; Zhou et al., 2011). The porous carbon materials, however, are generally insufficient in the electric conductivity and catalytic activity for the electrochemical reduction on cathode. The surface of porous carbon materials could be physicochemically and electrochemically modified with catalytic materials assisting electron transfer for a better performed cathode (Song et al., 2015b; Kadier et al., 2015; Dai et al., 2016). MWCNT is a material with highly conductive and wide surface area, and it is frequently adopted as a supporter for catalytic materials (Chen et al., 2015; Wang et al., 2016). Up to date, several substances, including cobalt tetramethylphenylporphyrin (CoTMPP), iron phthalocyanine (FePC), manganese oxide (MnOx), ammonia (NH_3), nickel (Ni), and platinum (Pt), have been examined as catalytic materials in bioelectrochemical systems (Wei et al., 2011; Zhou et al., 2011; Oliveira et al., 2013; Watson et al., 2013; Singh & Verma, 2015). Although Pt was always the best as the catalyst for the reductions on cathode, it was too expensive to use in the field scale (Ando et al., 2010; Yen et al., 2013). In recent, the reduction of carbon dioxide into methane on the cathode was successfully improved by some non-precious metals, such as nickel, copper and iron, as well as the nitrogen doping material using ammonia treatment (Cheng & Logan, 2007; Liu et al., 2010; Siegert et al., 2014; Sangeetha et al., 2016; Ullery et al., 2015). These imply that the porous carbon materials could be modified to a good cathode for

bioelectrochemical methane production by the surface decoration using above materials.

In this study, the electrochemical property improvement of the GFF were attempted by either of the pretreatments, the sonication or the electrophoretic deposition (EPD) using MWCNT and nickel, and the GFFs were further modified to complete the anodes by forming a scaffold layer with the paste of EG and MWCNT using either coal tar pitch or epoxy as the binding agent. It was examined how the anode modifications effect on the start-up and the performance of bioelectrochemical anaerobic digester. For the cathode study, three different cathodes were prepared by decorating with three catalytic materials (Ni, Fe and ammonia) together with MWCNT on the surface of GFF, and the bioelectrochemical methane production from the cathodes were examined in batch anaerobic reactor. The results of anode and cathode were published in 'Energy and Fuels' and 'Journal of applied electrochemistry', respectively (Feng & Song, 2016a,b).

3.2 Materials and methods

3.2.1 Anode for bioelectrochemical anaerobic digestion

A commercially available GFF (Samjung C&G Co., South Korea) and MWCNT (Carbon Nano-material technology Co., Ltd., South Korea) as basic electrode materials were submerged in concentrated nitric acid solution for 24 hours to improve their hydrophilicity and to remove impurities, and were then washed with tap water to remove excess nitric acid. The GFF surface was modified with MWCNT by either of a sonication or an EPD. For the sonication, the GFF was dipped into the solution containing 1 g MWCNT dispersed in 1 L distilled water, and then the MWCNT was loaded on the GFF surface using the sonication for 30min (Powersonic 420, 50/60Hz, 700W, HSt, South Korea). For the EPD, an electrolyte solution was prepared by dispersing 1g MWCNT, 0.25g nickel chloride, and 0.5g ployethylenimine into 1L distilled water. The GFF was submerged into the electrolyte solution,

and both of MWCNT and Ni were simultaneously deposited on the GFF surface by applying 30V for 30min using a direct current (DC) power source (OPM series, ODA Technologies Co. Ltd, Incheon, South Korea). The pretreated GFF sheet and the stainless steel mesh were used as the working electrode and the counter electrode, respectively.

The modified GFF was further processed by screen-printing with the pastes of EG and MWCNT to make a scaffold layer on the surface. The paste was prepared by mixing 1g of EG and 1g of MWCNT with 10mL of the binder. The binder was obtained by dissolving 2g of coal tar pitch into 10mL of toluene solution or dissolving 2g of epoxy into 10mL of ethanol solution. Finally, four different GFF anodes were fabricated as follows, i) S-CB: the GFF was pretreated by sonication in MWCNT solution and then the paste of EG and MWCNT with a coal tar pitch binder was screen-printed on the surface for the scaffold layer, ii) S-EB: the GFF was modified in the same procedure as S-CB, but the paste with the epoxy binder was used for the scaffold layer, iii) E-CB: the GFF was pretreated using the EPD method and the paste with the coal tar pitch binder was then screen-printed for the scaffold layer, and iv) E-EB: the GFF was modified using the same method as E-CB, but the paste with the epoxy binder was used for the scaffold layer. The GFF submerged in the nitric acid solution without further processing was also used as the CT-GFF anode. The GFF modified with MWCNT and nickel using the EPD method was used as the cathodes for all experiments. For the improvement of their hydrophilicity, all electrodes were submerged into 1% sodium dodecyl sulfate solution for 30 minutes and then washed with distilled water before the use, according to a previous study (Song et al., 2015a).

3.2.2 Cathode for bioelectrochemical anaerobic digestion

GFF (Samjung C&G Co., South Korea) and MWCNT were submerged in concentrated nitric acid for 24 hours to remove impurities, and then washed with running tap water. Different electrolyte solutions were prepared by

mixing 1.0 g MWCNT, 0.5 g ployethylenimine, and one of the electron transfer assisting materials including 0.25 g of $NiCl_2$, FePC, and NH3Cl with 1L of distilled water. The MWCNT and assisting materials were simultaneously loaded on each surface of the GFF by EPD method at 30V for 30 minutes, and three different cathodes, hereafter referred to as Ni-C, Fe-C, and Am-C, were obtained. The GFF without any treatment, referred to as GFF-C, was prepared as a control cathode. For the anode, the MWCNT and the Ni were loaded on the GFF surface by EPD method using the same procedure for the Ni-C preparation. A paste of EG and MWCNT was screen-printed to form a scaffold layer on the GFF surface, and then hot pressed for 15 minutes at 200℃ to complete the anode. For the EG, an acidified graphite powder soaked with diluted sulfuric acid and chromic acid (Hyundai Coma Industry, Inc., South Korea) were exfoliated by microwave radiation for 10 seconds, and then reduced using Hydrazine solution as in a previous study (Tang et al., 2015). The paste of EG and MWCNT was obtained by mixing 1 g of MWCNT, 1 g of EG, 100 mL of ethanol, and 10mL of binder. The binder was prepared by dissolving 2g of coal tar pitch into 10mL of toluene. The anode and cathode were submerged into a sodium dodecyl sulfate (SDS) solution (1%) to improve the hydrophilicity before use according to a previous study (Feng & Song, 2016a).

3.2.3 Reactor Start-up and operation

A separator and electrodes assembly (SEA) was prepared by stacking in order of the anode, the polypropylene sheet as a separator, and the cathode. The SEA was rolled to a cylinder shape (6 cm diameter, 8 cm height) and installed inside a glass bottle anaerobic reactor (effective volume 0.75L) (Fig. 3.1). The anode and the cathode were connected to a DC power supply (OPM series, ODA Technologies Co. Ltd, South Korea) using a titanium wire as current collector. The anaerobic reactor was covered with an airtight cap, and a biogas venting port and a gas sampling port were installed on the cap.

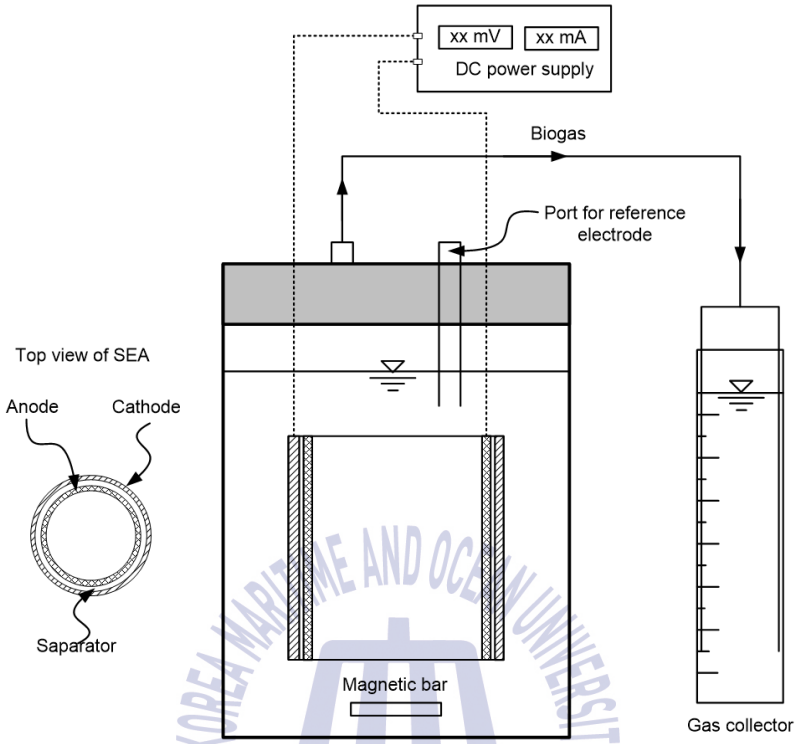


Fig. 3.1. Schematic diagram of batch bioelectrochemical anaerobic reactor.

The gas venting port was connected to a floating type gas collector using a rubber tube. The gas collector was filled with an acidified solution saturated with sodium chloride to minimize the dissolution of biogas (Walker et al., 2009). The gas sampling port was covered with a butyl rubber stopper. For the start-up, a medium solution (0.525 L) was added to the reactor, and then inoculated with seed sludge. The medium containing 3 g/L glucose, 2.45 g/L NaH_2PO_4, 4.58g/L Na_2HPO_4, 0.31g/L NH_4Cl, 0.13g/L KCl, trace vitamins, and minerals was prepared, according to previous studies (Balch et al., 1979; Ullery et al., 2015). The seed sludge was taken from an anaerobic digester for sewage sludge (B metropolitan, South Korea). The liquid in the bottle reactor was then mixed using a magnetic bar at 300 rpm, continuously, and the potential difference between the anode and the cathode was maintained at 0.3V by using the DC power supply. The prepared bottle reactor was then

incubated in a temperature controlled room at 35 ℃. During the operation, the available substrate depletion in the anaerobic batch reactor was estimated from the biogas production, and the medium was then replaced with a fresh medium after settling suspended sludge for 30 minutes. As an anaerobic control, a reactor without electrodes was also incubated in the same condition. The bioelectrochemical anaerobic reactor was operated in a semi batch mode during 5 cycles (92days).

3.2.4 Properties of electrodes and electrochemical measurement

The ohmic resistance for the surface modified anodes was measured using a hall measurement system (Ecopia HMS-3000), and the surface feature was observed using a scanning electron microscope (SEM, MIRA-3, Tescan, Czech). The elemental compositions of the anode surfaces were analyzed using energy dispersive X-ray spectroscopy (EDS) based on scanning electron microscopy (SEM). During the operation of the bioelectrochemical anaerobic batch reactor, the potentials of the anode and the cathode were frequently checked with a portable digital multimeter (Fluke 87-V) with an Ag/AgCl reference electrode. An electrochemical impedance spectroscopy (EIS) test was performed on the electrodes with an Ag/AgCl reference electrode. A potential wave signal (25mV) was applied on the electrode in the frequency ranging from 100 kHz to 10 MHz, and the impedance responses were fitted by using Iviumstat analysis software, into a Randles equivalent circuit model with mixed kinetic and diffusion control. It includes a solution ohmic resistance in series with a double-layer capacitor, which is in parallel with the Faradic reaction impedance consisting of a charge transfer resistance and Warburg element in series (Liang et al., 2011). A cyclic voltammetry (CV) test for the anode was also conducted with a potential range of between -200mV and 650mV. An Ag/AgCl electrode served as the reference electrode and the scan rate was 10mV/s (Sakai et al., 2009; Sun et al., 2011). The polarization data was fitted into Equation 3.1, and Tafel slope (β) and exchange current (i_0) were obtained from the extrapolation of the linear region ($r>0.999$).

Equation 3.1: $E = \alpha + \beta \log i/i_0$

where E is the potential ($E_0 + \eta$), α and i are the intercept of the Tafel curve and the anodic current density, and η and E0 are the overpotential and the equilibrium potential.

3.2.5 Chemical analysis and calculation

During the operation of the bioelectrochemical anaerobic batch reactors, the reactor contents were frequently taken out and the changes of chemical properties such as pH and COD were analyzed. Here, pH was measured with a pH meter (Orion 370), and COD was determined by the closed reflux method in the Standard methods. The biogas production was periodically monitored from the gas collector. The biogas composition was analyzed using a gas chromatograph (GowMac Series 580, Porpak Q 6ft × 1/8 "SS) equipped with a thermal conductivity detector (TCD). Nitrogen gas (30ml/min flow rate) was used for the moving phase. The temperatures of the injector, detector, and column were 80, 90, 50°C, respectively. The biogas production was converted into the standard temperature and pressure (STP) state using equation 3.2:

Equation 3.2: $V_{CH_4}(STP\ L) = V_{CH_4}(at\ T) \times \frac{273}{273+T} \times \frac{760-W}{760}$

Where T is the operation temperature (35 ℃) of the batch reactor and W is the water vapor pressure at 35℃ (mm Hg). The methane production (V_{CH_4}) at each monitoring time interval was calculated from the measurements of the biogas volume and their methane contents in the headspace and the gas collector using the following mass balance equation (3) (Woo et al., 2010).

Equation 3.3: $V_{CH_4,i} = C_{CH_4,i}(V_{G,i} + V_R) - V_R C_{CH_4,i-1}$

Where $V_{G,i}$ is the total biogas volume (mL) measured in the gas collector at the current time interval (i) and V_R is the headspace volume in the bottle reactor. $C_{CH4,i}$ and $C_{CH4,i-1}$ are the methane contents in the headspace of the

reactor measured using gas chromatography in current and previous monitoring time intervals, respectively. As the cumulative methane production from a batch cycle operation was similar to that in the following batch cycle, it was considered that the EAB on the electrode was maturated, and the average cumulative methane production was used for fitting into the Modified Gompertz Equation 3.4 (Woo et al., 2010),

Equation 3.4: $$P = P_u \exp\left[-\exp\left(\frac{\mu_m e}{P_u}(\lambda - t) + 1\right)\right]$$

Where μ_m is the maximum methane production rate (mL/L.d), λ is the lag phase time (days), and P_u is the ultimate methane production (mL/L).

3.3 Results and discussion

3.3.1 Influence of anode on bioelectrochemical anaerobic digestion

① Properties of surface modified GFF anodes

The GFF sheet treated with nitric acid was consisted of carbon fiber strands with clean and smooth surface in SEM image (Fig. 3.2a-inset), and the electric resistance was quite high as 7.52Ω/cm. However, the GFF surface was changed to a little rough plane layer by the EPD treatment, and the carbon fiber strands were disappeared (Fig. 3.2a). In the EDS data, the main components of the EPD treated layer were carbon (45.85 % At.), oxygen (37.17 % At.) and nickel (16.67 % At.) (Table 3.1). The electric resistance of the EPD treated GFF (E-GFF) was considerably reduced to 0.0125Ω/cm. This demonstrates that the EPD treatment is a good approach for the loading of MWCNT and Ni simultaneously on the GFF surface. The improved conductivity of the E-GFF is possibly attributed to the amount of highly conductive MWCNT (17~2.0×10^8 S/m) and nickel (1.45×10^7 S/m) loaded on the surface (Song et al., 2016). On the other hand, when the GFF was

sonicated in MWCNT solution, the MWCNT was rarely and irregularly observed on the carbon fiber surface, but there are some accumulations of MWCNT between the carbon fiber strands (Fig. 3.2b). The electric resistance of the sonicated GFF (S-GFF) was also reduced to 0.278 Ω/cm, although it was slightly higher than the E-GFF.

In the case of the modified GFF anodes, the thick scaffold layer was observed on the surface. It is believed that the scaffold layer on the anode surfaces was formed by screen-printing the paste of EG and MWCNT. However, the morphologies of the scaffold layer were quite different depending on the binding agents of the paste in SEM images. The scaffold layer surface of C-GFF anodes (S-CB, E-CB), in which the coal tar pitch was used as the binding agent for the paste of EG and MWCNT, was rough and porous, but somewhat compact (Fig. 3.2c, e), demonstrating that the coal tar pitch is a good adhesive binding agent. The rough and porous surface of the solid material is commonly favorable for bacterial attachment and growth (Katsikogianni et al., 2004). The surface of the scaffold layers on the E-GFF anodes (S-EB, E-EB) using epoxy binder, in contrast, were a little loose, and partially bare carbon fiber bundles were also observed on the surface (Fig. 3.2d, f). It seems that the adhesive force of the epoxy is insufficient for the paste of EG and MWCNT, compared to the coal tar pitch. The elemental composition of the modified GFF anode surface was affected by the pretreatments as well as, the binders for the scaffold layer (Table 3.1). The main component for the S-GFF anodes (S-CB, S-EB) was carbon (86.5-92.0%). In the surface of the E-GFF anodes (E-CB, E-EB), the carbon contents were reduced to 70.0-72.7%, but the nickel contents were as high as 9.7-10.9%. The high Ni content of the E-GFF anodes (E-CB, E-EB) are probably due to the pores and cracks on the scaffold layer (Fig. 3.2 e, f). For the whole GFF anodes, the electric resistances were ranged from 0.16 to 0.39Ω/cm (Table 3.1), which were higher than those of the GFFs pretreated by either of the EPD or the sonication only. This indicates that the electric conductivity of the surface modified GFF anodes was considerably governed

by the scaffold layer.

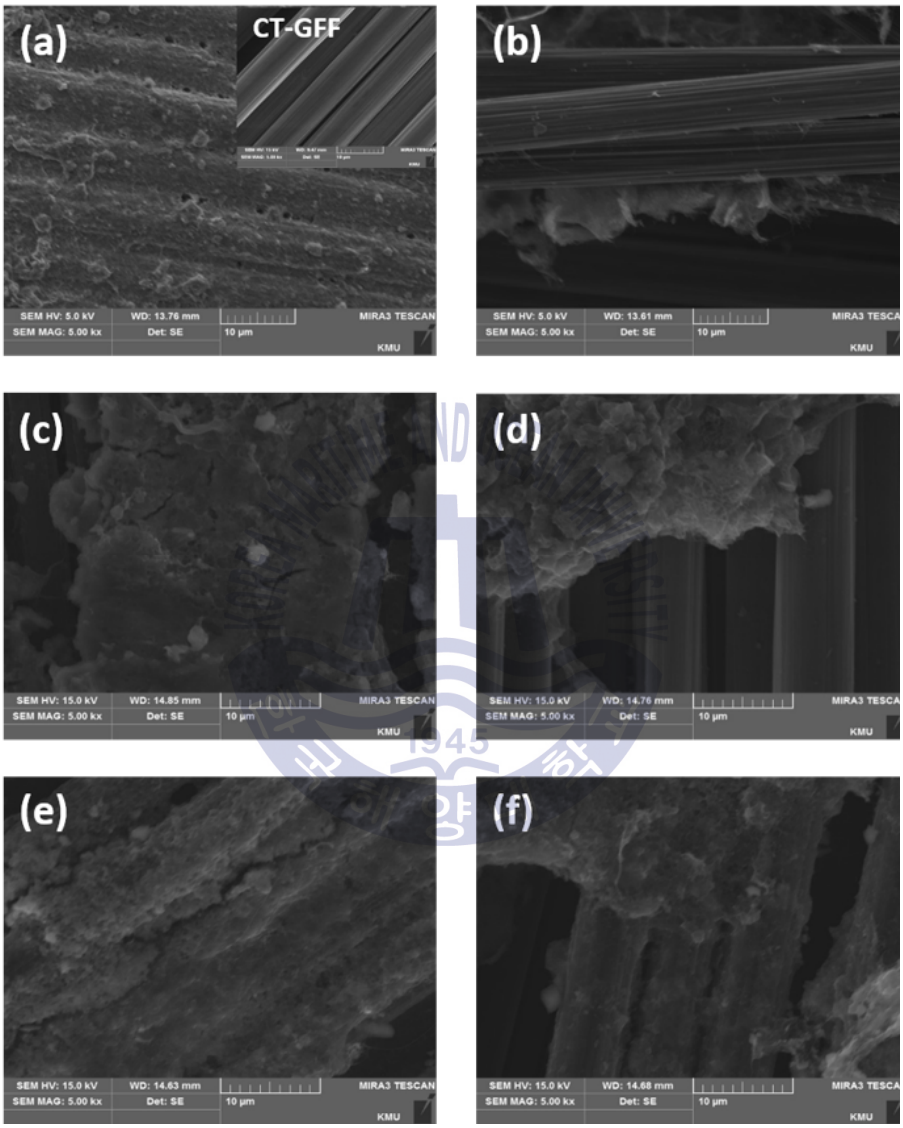


Fig. 3.2. Scanning electron microscopy (SEM) images for surface modified GFFs (a) E-GFF (inset-CT), (b) S-GFF, (c) S-CB, (d) S-EB, (e) E-CB, (f) E-EB.

Table 3.1 EDS data for element components and electrical resistances for surface modified anodes

Element (At %)	S-GFF	E-GFF	S-CB	S-EB	E-CB	E-EB	CT-GFF
C	100	45.85	86.47	91.95	70.07	72.74	89.48
O	-	37.17	11.41	5.91	17.39	16.85	10.52
Cl	-	-	0.91	1.37	1.94	0.68	-
Ni	-	16.97	-	-	10.88	9.72	-
S	-	-	1.21	0.77	-	-	-
Resistance (Ω/cm)	0.278	0.0125	0.29	0.18	0.16	0.39	7.52

② Start-up of bioelectrochemical anaerobic reactor

The cumulative methane productions in the bioelectrochemical digesters were gradually increased after long lag times (Fig. 3.3). However, there were some differences in the methane production features between the bioelectrochemical digesters with different anodes. For the E-GFF anodes (E-CB, E-EB), the lag times in the methane production were 20.8-23.3 days, which were clearly longer than 17.1-18.4 days for the S-GFF anodes (S-CB, S-EB) (Table 3.2). The lag time in methane production is defined as the time required for the initial adhesion of the inoculated bacteria on the electrode surface, and then the selection and adaptation for the subsequent colonization of the bacteria (Parot et al., 2008; Baudler et al., 2014). The initial adhesion of bacteria depends on the physicochemical characteristics of the electrode surface, such as biocompatibility, roughness, porosity, surface functional group and configuration (Katsikogianni et al., 2004; Diaz et al., 2007), whereas the selection and adaptation of EAB on the anode surface are mainly governed by the electrochemical properties, such as electric conductivity and catalytic activity for electron transfer (Song et al., 2015a). As mentioned earlier (Fig. 3.2), there were no considerable differences in the physical properties of the surfaces between the E-GFF anodes (E-CB, E-EB) and the S-GFF anodes (S-CB, S-EB), but the surface of the E-GFF anodes (E-CB, E-EB) contained about 10% of Ni component. It is reported that some Ni compounds have a

catalytic activity for electron transfer reaction (Amatore & Jutand, 1990; Martinez-Perinan et al., 2015; Pan et al., 2015), indicating that the electrochemical properties of the E-GFF anodes (E-CB, E-EB) are better than the S-GFF anodes (S-CB, S-EB). It is probably that EAB is more strictly selected and adapted on the EPD treated GFF anodes, leading to longer lag time for the initial methane production, rather than the sonication treated GFF anodes. It is unlikely that the lag phase was caused by the toxicity of the raw materials including MWCNT, Ni and binders to EAB. It is commonly recognized that the MWCNT loaded on the electrode plays an important role in the electron transfer of electroactive bacteria (EAB) rather than the toxic to EAB (Song et al., 2015a), and the elemental metals including Ni have catalytic activity on the bioelectrochemical reaction38. The binders such as coal tar pitch and epoxy are also non-toxic materials to bacteria. The lag time for the CT-GFF anode was 15.52 days, which was shorter than others due to the low electric conductivity of the GFF. In the case of the control anaerobic reactor, the lag time was only 6.9 days. It seems than the anaerobic bacteria in the control reactor does not require any additional time to adhere on the anode surface and colonize subsequently due to the lack of electrochemical properties, but only short time is enough for the adaption to new environment after the inoculation. Then, the lag time was also affected by the binding agent used for the scaffold layer of the GFF anode. The lag time for the methane production were 17.1-20.8 days for the CB-GFF anodes (S-CB, E-CB), which were shorter than those for the EB-GFF anodes (S-EB, E-EB). It is likely that the coal tar pitch binder provides more favorable environment for the initial adhesion of the inoculated bacteria rather than the epoxy binder.

The maximum methane production rate was also affected by the surface modifications of the GFF anode during the start-up period (Fig. 3.3). An important methane source in bioelectrochemical anaerobic digester is the bioelectrochemical conversion of carbon dioxide into methane (Villano et al., 2011; Sun et al., 2011; Koch, et al., 2015; Zhao et al., 2015; Song et al.,

2016). In a bioelectrochemical anaerobic reactor, the organic substance is hydrolyzed and acidified into volatile fatty acids (VFAs) by the planktonic acidogens. The VFAs are oxidized on anode surface into carbon dioxide, and the methane is produced by the reduction of the carbon dioxide on the cathode (Guo et al., 2013; Koch et al., 2015; Feng et al., 2015). This indicates that the bioelectrochemical methane production is directly coupled with the oxidation of organic fatty acids on the anode. Another important methane sources are the syntrophic methanogens rather than the acetoclastic methanogens3,40. It is reported that the electron transfer of the syntrophic bacteria is enhanced by the electron transfer mediator (Villano et al., 2011; Richter et al., 2012; Shrestha et al., 2014; Sun et al., 2015). Then, the mediator is secreted from the EAB on the electrode surface (Marsili et al., 2008). This implies that higher methane production rate is closely connected to higher bioelectrochemical activity of the electrode. In the present study, the maximum methane production rates was 5.4-8.5 mL CH_4/g COD.d for the E-GFF anodes (E-EB, E-CB), which were higher than those of the S-GFF anodes (S-EB, S-CB) (Table 3.2). It is believed that the maximum methane production rate of the E-GFF anode was improved by the electrochemical catalytic activity of Ni compounds (Amatore & Jutand, 1990; Martinez-Perinan et al., 2015). The maximum methane production rate was also affected by the binder type used for the scaffold layer of the surface modified anodes. In the case of the CB-GFF anodes (S-CB, E-CB), the maximum methane production rates were 4.9-8.5 mL CH_4/g COD.d, which were higher than those for the EB-GFF anodes (S-EB, E-EB). The discrepancy between the lag time and the maximum methane production rate demonstrates the above hypothesis that the electroactive electrode requires a long lag time for the strict selection and adaption of EAB. The highest value of the maximum methane production rate was obtained from the E-CB GFF anode. It seems that the highest bioelectrochemical activity for the E-CB GFF anode is attributed to both of the superior electrochemical properties of the anode containing Ni compound and the biocompatibility of the coal tar pitch binder for the scaffold layer.

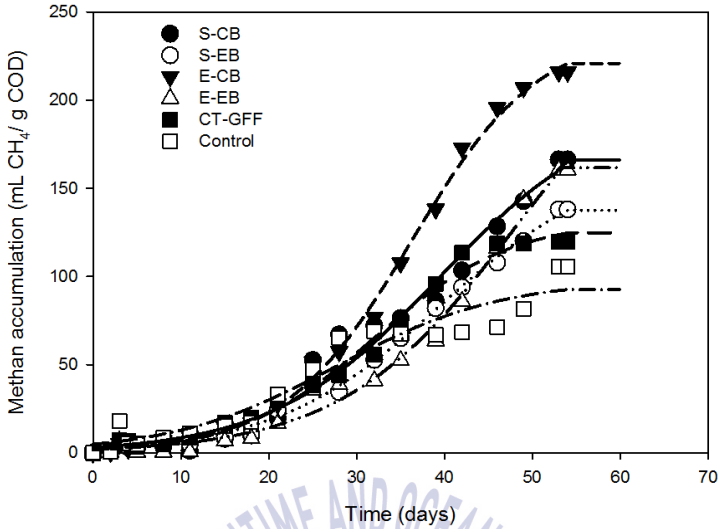


Fig. 3.3. Cumulative methane productions from the bioelectrochemical reactors with surface modified anodes and the control anaerobic reactor.

Table 3.2 Summary of the lag time, the maximum methane production rate, and the ultimate methane production for the surface modified GFF anodes estimated from Modified Gompertz equation

Parameter	Period	S-CB	S-EB	E-CB	E-EB	CT-GFF	Control
P_u ($mLCH_4/g$ COD)	S	179.5	148.4	226.1	174.9	126	114.2
	M	195.8	148.2	224.1	167.7	131.8	89.2
μ_m ($mLCH_4/g$ COD.d)	S	4.9	4.3	8.5	5.4	4.3	2.4
	M	35.8	33.1	47.4	30.1	22.8	17.8
λ (d)	S	17.14	18.36	20.78	23.32	15.52	6.90
	M	0.44	0.30	0.20	0.45	0.70	1.53
r^2	S	0.985	0.990	0.990	0.977	0.981	0.965
	M	0.997	0.995	0.994	0.996	0.996	0.985
Methane Yield ($mLCH_4/gCOD_r$)	M	280.2	230.6	322.9	261.0	211.0	162.1

(S: start-up period, M: after EAB matured)

③ Methane production from the matured bioelectrochemical reactor

From the 2nd batch cycle of the batch bioelectrochemical anaerobic reactor, the methane was immediately produced when the substrate depleted medium was replaced with fresh one (Fig. 3.4). A common response of microorganisms to the substrate depletion is the adaptive changes of the biochemical- and physiological reactions to reduce the metabolism, but the microorganisms recover their metabolic activity when the substrate is available (Kjelleberg et al., 1993). The lag time for methane production in the batch cycle is an indicator for the recovery of the microbial metabolic activity in anaerobic batch reactor. In the present study, the cumulative methane production for the 3rd batch cycle was similar to the following batch cycles (Fig. 3.4), indicating that EAB on the electrode surface was sufficiently matured. For the surface modified GFF anodes, the lag times estimated from the 3rd to the 5th batch cycles were 0.2-0.7 days for the bioelectrochemical methane productions, which were shorter than 1.53 days for the anaerobic control (Table 3.2). This indicates that the metabolic activity recovery of the EAB is possibly faster than the planktonic bacteria when the substrate depleted medium was replaced with fresh one. The quick response in the metabolic activity is an important physiological characteristic of EAB, which is often observed in batch microbial fuel cell (Song et al., 2015b).

The maximum methane production rate was also affected by the surface modifications of the GFF anode during the start-up period (Fig. 3.3). An important methane source in bioelectrochemical anaerobic digester is the bioelectrochemical conversion of carbon dioxide into methane (Villano et al., 2011; Sun et al., 2011; Koch, et al., 2015; Zhao et al., 2015; Song et al., 2016). In a bioelectrochemical anaerobic reactor, the organic substance is hydrolyzed and acidified into volatile fatty acids (VFAs) by the planktonic acidogens. The VFAs are oxidized on anode surface into carbon dioxide, and the methane is produced by the reduction of the carbon dioxide on the cathode (Guo et al., 2013; Koch et al., 2015; Feng et al., 2015). This indicates that the bioelectrochemical methane production is directly coupled

with the oxidation of organic fatty acids on the anode. Another important methane sources are the syntrophic methanogens rather than the acetoclastic methanogens3,40. It is reported that the electron transfer of the syntrophic bacteria is enhanced by the electron transfer mediator (Villano et al., 2011; Richter et al., 2012; Shrestha et al., 2014; Sun et al., 2015). Then, the mediator is secreted from the EAB on the electrode surface (Marsili et al., 2008). This implies that higher methane production rate is closely connected to higher bioelectrochemical activity of the electrode. In the present study, the maximum methane production rates was 5.4-8.5 mL CH_4/g COD.d for the E-GFF anodes (E-EB, E-CB), which were higher than those of the S-GFF anodes (S-EB, S-CB) (Table 3.2). It is believed that the maximum methane production rate of the E-GFF anode was improved by the electrochemical catalytic activity of Ni compounds (Amatore & Jutand, 1990; Martinez-Perinan et al., 2015). The maximum methane production rate was also affected by the binder type used for the scaffold layer of the surface modified anodes. In the case of the CB-GFF anodes (S-CB, E-CB), the maximum methane production rates were 4.9-8.5 mL CH_4/g COD.d, which were higher than those for the EB-GFF anodes (S-EB, E-EB). The discrepancy between the lag time and the maximum methane production rate demonstrates the above hypothesis that the electroactive electrode requires a long lag time for the strict selection and adaption of EAB. The highest value of the maximum methane production rate was obtained from the E-CB GFF anode. It seems that the highest bioelectrochemical activity for the E-CB GFF anode is attributed to both of the superior electrochemical properties of the anode containing Ni compound and the biocompatibility of the coal tar pitch binder for the scaffold layer.

Both the ultimate methane production and methane yield were also affected by the surface modifications for GFF anode. The highest values of the ultimate methane production and the methane yield were 224.1 mL CH_4/g COD and 322.9 mL CH_4/g COD_r, respectively, which were obtained from the E-CB GFF anode (Table 3.2). The methane yield of the control anaerobic

reactor was only 162.1 mL CH_4/g COD. The methane yield is an indicator for the energy recovery during the bioelectrochemical conversion of organic matter to methane. The energy loss for methane production could be described by the overpotentials in the bioelectrochemical system (Clauwaert et al., 2008; Song et al., 2016). The bioelectrochemical methane yield for the CT-GFF anode was as small as 211.0 mL CH_4/g COD, indicating higher energy losses, compared to those for the other surface modified anodes. These indicate that the energy efficiency of the bioelectrochemical anaerobic digestion depends on the electrochemical properties of anode, as well as the physical properties for bacterial growth.

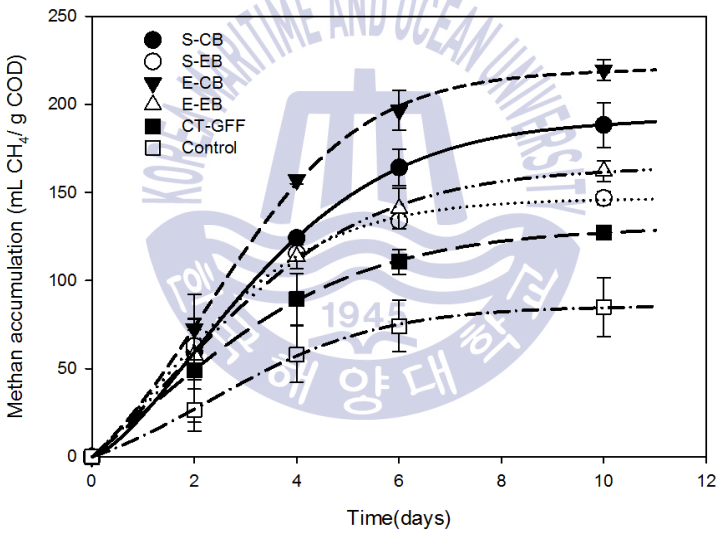


Fig. 3.4. Average cumulative methane production for the matured bioelectrochemical reactors with surface modified anodes and the control reactor.

④ Electrochemical characterization for surface modified anodes

The EIS data in the Nyquist representation were fitted well by a Randles equivalent circuit model (Fig. 3.5a). This suggests that bioelectrochemical methane production was mainly controlled by charge transfer, and partly by

diffusion on the anode surface. The estimated values of Rs, Rct, W, and C were presented in Table 3.3. The solution ohmic resistances for the surface modified anodes were in the range of 1.4-1.7 Ω, which were similar to 2.1 Ω of the CT-GFF anode. The semi-circle diameter of Nyquist plot indicates the charge transfer resistance (Yamada et al., 2010; Ishihara et al., 2014). The charge transfer resistance was considerably affected by the surface modifications of GFF anode. For the E-GFF anodes (E-CB, E-EB), the charge transfer resistances were 5.4-13.4 Ω, which were smaller than those for the S-GFF anodes (S-CB, S-EB). Especially, the charge transfer resistance was only 5.4 Ω for the E-CB GFF anode, which was considerably smaller than that of the E-EB anode. These results are in agreement with the methane production features (ultimate methane production and maximum methane production rate) which were higher for the E-GFF anodes (E-CB, E-EB) than those for the S-GFF anodes (S-CB, S-EB) (Fig. 3.3). For the different binder anodes, there are some relationship between the lag time and the charge transfer resistance. In the case of the coal tar pitch binder anodes (S-CB, E-CB), the charge transfer resistance was 29.7-59.7% smaller than that of the epoxy binder anodes (S-EB, E-EB) (Table 3.3). The lag time was shorter for the coal tar pitch binder anodes (S-CB, E-CB) than the epoxy binder anodes (S-EB, E-EB) (Table 3.2). This indicates that the coal tar pitch binder is more biocompatible than the epoxy binder. It seems that the initial adhesion is mainly affected by the biocompatibility of the electrode, and the selection and adaptation of EAB is governed by the electrochemical properties. The charge transfer resistance for the CT-GFF anode without surface modification was 27.4Ω. The activation energy for the bioelectrochemical reaction on the electrode can be expressed with a function of the charge transfer resistance using the Arrhenius equation (Yamada et al., 2010; Ishihara et al., 2014). This indicates that the electron transfer reactions for bioelectrochemical methane production from organic matter on the electrode are catalyzed by the EAB (Song et al., 2015a). The smaller charge transfer resistance suggests that the electrode has a kinetic advantage for bioelectrochemical reaction.

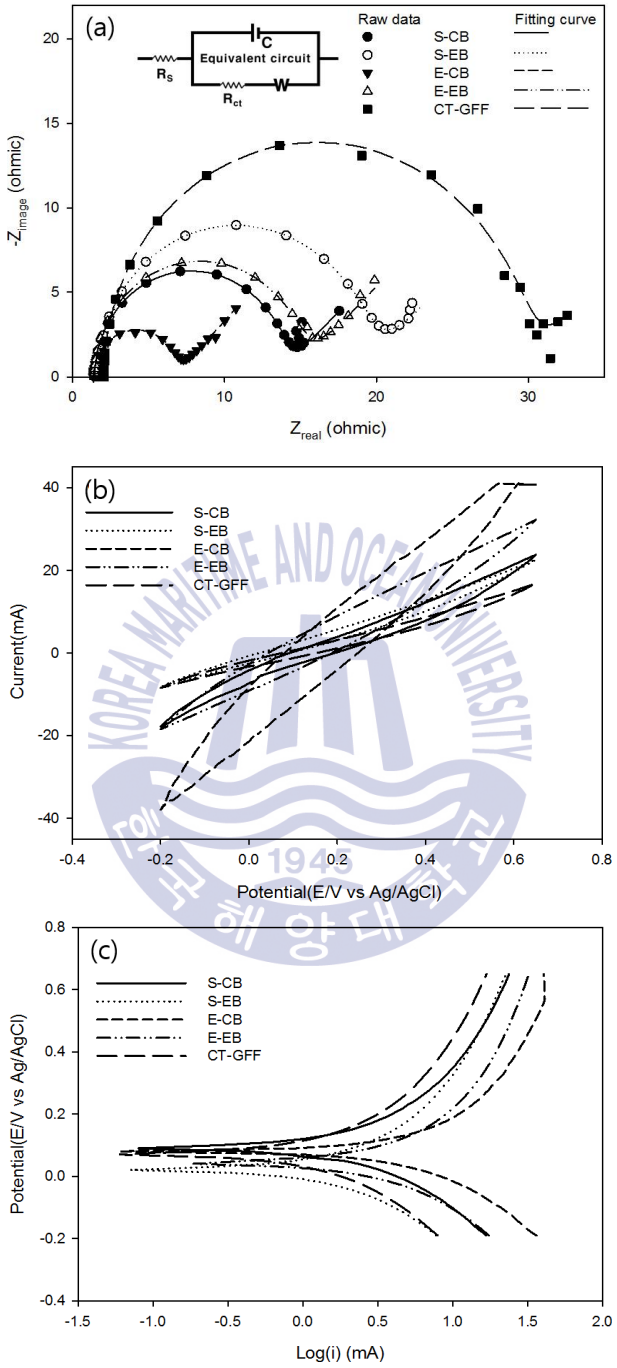


Fig. 3.5. (a) Nyquist plots, (b) CV and (c) Tafel plot for surface modified anodes in bioelectrochemical anaerobic reactor.

Table 3.3 Electrochemical properties for surface modified anodes estimated from EIS and Tafel plot

Contents	S-CB	S-EB	E-CB	E-EB	CT-GFF
Rs (Ω)	1.452	1.651	1.365	1.442	2.054
Rct (Ω)	12.3	17.5	5.4	13.4	27.4
C (mF)	4.9	6.8	4.5	4.8	8.0
W ($1/\Omega\sqrt{Hz}$)	0.9583	1.1440	1.0090	1.2860	0.7944
β_a (mV/dec)	225	240	185	269	220
i_0 (mA)	2.03	1.89	2.65	2.46	1.33

In the case of double layer capacitance, the CT-GFF anode was 8.0 mF, but reduced to 4.5-6.8 mF for the surface modified anodes (Table 3.3). Then, the double layer capacitances were slightly lower for either the EPD treated or the coal tar pitch binder than those for the sonication treated or the epoxy binder anodes. The Warburg impedance represents the diffusion resistance on electrode surface, depending on the surface geometry. The Warburg impedance of the CT-GFF anode was 0.79 Ω, which was slightly increased to 0.96-1.14 Ω by the surface modifications of the anodes (Table 3.3), reflected that the surfaces of the modified GFF anodes are more porous and complex. These indicate that the bioelectrochemical catalytic activity of the surface modified anode is mainly enhanced by the reduced charge transfer resistance. The bioelectrochemical catalytic activities of the surface modified anodes were confirmed again by CV and Tafel slope (Fig. 3.5b, c). Although it was difficult to check the oxidation peaks from the CVs at a turnover condition, the currents patterns responding the voltage scan were quite different depending on the surface modification methods of the GFF anodes. For example, the highest anodic current at -0.1V was obtained from the E-CB GFF anode, and the anodic currents were slightly higher for either anodes of the EPD treated or the coal tar pitch binder used than the others. Higher anodic current in CV indicates higher catalytic activity of the anode.

Tafel slope and exchange current for the surface modified anodes give insight

into the bioelectrochemical reaction. As well known, smaller Tafel slope and higher exchange current indicate higher catalytic activity and smaller activation energy for the electrochemical reaction on the electrode (Mansfeld et al., 2005; Hou et al., 2007). In the present study, the smallest Tafel slope was 185mV/dec, which was obtained from the E-CB GFF anode, and followed by the CT-GFF anode of 220mV/dec (Table 3.3). The EB GFF anodes (S-EB, E-EB) show higher Tafel slope than those of the CB GFF anodes (S-CB, E-CB), reflecting that the epoxy binder for the scaffold layer is a non-conductive and less biocompatible polymer. The bioelectrochemical catalytic activity depends on the dominant species of EAB, as well as the EAB biomass on the electrode (Sun et al., 2015; Ullery et al., 2015). However, in the case of the exchange current, the highest value was 2.65 mA, which was also obtained from the E-CB GFF anode. The exchange current for the E-EB GFF anode (EE) was as high as 2.46 mA, which was possibly attributed to the catalytic activity of Ni compound. Then, the exchange current for the CT-GFF anode was 1.33 mA, which was smaller than others, probably due to high ohmic resistance of the GFF. The low biochemical activity of the CT-GFF anode for methane production is ascribed to the low exchange current.

3.3.2 Influence of cathode on bioelectrochemical anaerobic digestion

① Morphology of the cathode surfaces

Fig. 3.6 displays SEM images for the cathode surfaces decorated with different materials assisting the electron transfer of EAB. The crystalline aggregations of the materials deposited on the graphite fiber strands appeared on the decorated cathode surfaces, which were porous and rough. The GFF-C without any decoration consisted of clean and smooth strands of carbon fibers. In bioelectrochemical methane production the reduction of carbon dioxide into methane is catalyzed by the EAB, which is growing on the cathode surface (Cheng et al., 2009; Jiang et al., 2013; Feng et al., 2015). It

is likely that the materials decorated on the cathode surface support the growth of EAB by assisting electron transfer on the cathode, as well as, altering the cathode surface to a more biocompatible and porous structure, which are favorable conditions for bacterial growth (Song et al., 2016; Tsai et al., 2009; Wei et al., 2011).

The elemental composition of the cathode surfaces was further examined by EDS (Table 3.4). The major elemental components of the decorated cathodes were carbon (74.7-87.6 At.%) and oxygen (7.3-20.9 At.%), while a small portion of the assisting materials were also appeared on the cathodes. This indicates that the crystalline aggregations on the cathode surface, the assisting materials, were properly decorated on the cathode surfaces by the EPD method. The percentage of the assisting materials appearing were in the range of 0.5-2.3 At.%, which was varied in the types of the materials. The GFF-C as the control cathode consisted of carbon (89.5 At.%) and oxygen (10.5 At.%).

The electric resistance for the GFF-C was observed as high as 7.52 Ω cm-1 (Table 3.4). The high resistance of the GFF-C was likely due to the contact resistance between the loose bundles of the carbon fiber strands. The electric resistances for the cathodes decorated with the assisting materials were considerably reduced, and in particular, the electric resistance for the Ni-C was 0.07 Ω cm-1, which was slightly lower than the others. The resistance for the Am-C decorated with non-conductive ammonia compound was also low, namely 0.15 Ω cm-1. This indicates that the improved electric conductivities for the decorated cathodes were mainly attributed to the MWCNT, which was loaded on the surface of the carbon fiber strands, and was bridged between the carbon fiber strands. The MWCNT is often used as a conducting bridge for the improvement of electric conductivity for various composites (Kim & Park, 2012; Kalakonda et al., 2015).

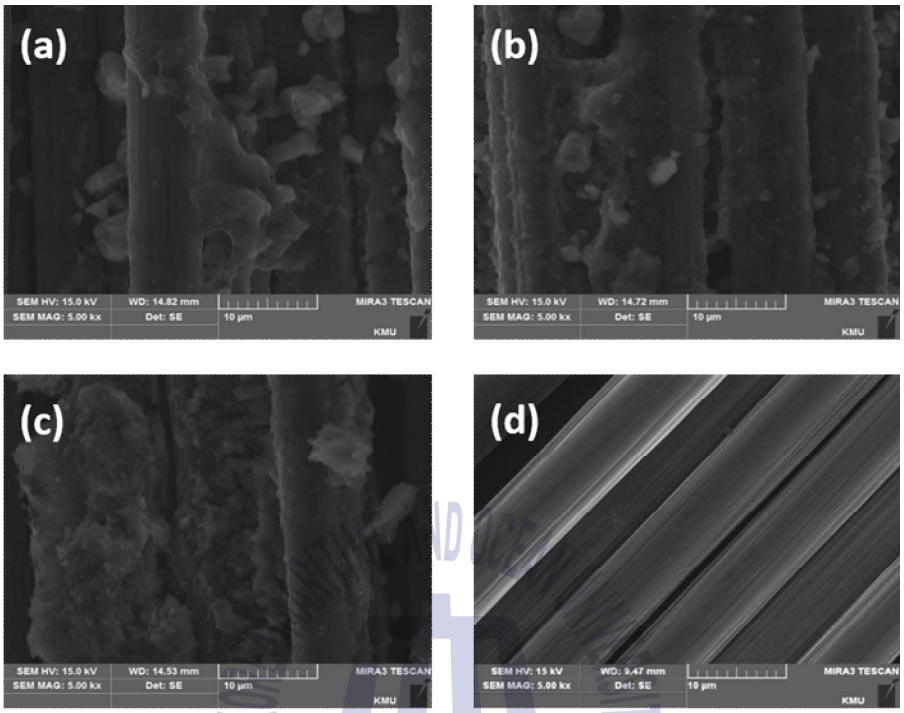


Fig. 3.6. SEM images for the different cathode surfaces decorated with the materials assisting with the electron transfer: a) Ni-C, b) Fe-C, c) Am-C, and d) GFF-C.

Table 3.4 Elemental compositions and electrical conductivities for different cathode surfaces

Element (At. %)	Ni-C	Fe-C	Am-C	GFF-C
C(%)	87.6	77.2	74.7	89.5
O(%)	7.3	20.9	14.2	10.5
Cl(%)	2.9	1.4	9.3	
Ni(%)	2.3	-	-	-
Fe(%)	-	0.5	-	-
N(%)	-	-	1.8	-
Resistance(Ω/cm)	0.07	0.19	0.15	7.52

② Enrichment stage of EAB on the decorated cathodes

The bioelectrochemical methane production during the enrichment stage of EAB on the cathode surface was considerably affected by the decoration materials (Ni, Fe, or ammonia) (Fig. 3.7). Methane production for the decorated cathodes started to increase slowly after initial lag phases of 9.4-22.5 days, but the lag phases for the GFF-C without any decoration and the anaerobic digestion control were only 13.0 and 6.9 days, respectively (Table 3.5). The long lag phase in the bioelectrochemical system is due to the time for the initial selection of EAB and adaptation to the new environment before beginning exponential growth (Parot et al., 2008;Rolfe et al., 2012).The lag phase depends on the seed sludge, the roughness and biocompatibility of the electrode surface, as well as, electrochemical properties (Feng & Song, 2016a; Rolfe et al., 2012). It seems that the initial attachment of bacteria on the electrode surface is affected by the types of seed sludge and the electrode physical properties, such as its roughness and biocompatibility, and the selection and adaption of EAB on the electrode surface depend on the electrochemical properties of the surface. The long lag phases for bioelectrochemical methane production compared to the control are most likely due to the seed sludge taken from an anaerobic digester for sewage sludge. The dominant species in the anaerobic sludge from a conventional anaerobic digester for sewage sludge are not EAB. This indicates that the materials (Ni, Fe, and ammonia) decorated on the cathode surface do not directly catalyze the electrochemical conversion of carbon dioxide into methane within conditions for mesophilic anaerobic digestion. The lag phase for the Am-C was shorter (9.38 days) than the GFF-C. This suggests that the biocompatibility on the cathode surface is improved by the ammonia treatment. In previous study, the ammonia treatment for the electrode improved bacterial attachment on the surface, and then the initial acclimation time of EAB was reduced in microbial fuel cell (Cheng & Logan, 2007). However, the lag phases for Ni-C and Fe-C were over 20days, which were considerably longer than 13 days of the lag phase for the GFF-C

(Table 3.5). This suggests that the selection and adaptation of EAB is stricter on the surface that is more electroactive, which caused a longer time for enrichment stage but not the inhibitory effects of decorated materials. In previous studies, it took a long enrichment time (10-30 days) of EAB on the surface of electroactive electrode after the inoculation of anaerobic sludge for sewage sludge (Yoon et al, 2007; Parot et la., 2008; Song et al., 2016).

The maximum methane production rate provides insight into the growth of EAB on the cathode surface. The maximum methane production rate was 6.2 mL CH_4 /g COD.d for the Ni-C, and 5.4 mL CH_4/g COD.d for the Fe-C. The maximum methane production rate of the Am-C was 3.6 mL CH_4/g COD.d, and similarly for the GFF-C (3.7 mL CH_4 /g COD.d). The methane in the bioelectrochemical anaerobic reactor is a main product from the metabolic process of electroactive methanogens. This implies that the growth rate of the methanogens could be inferred from the maximum methane production rate during log growth phase in batch reactor. It seems that the growth rate of EAB is the highest on the Ni-C, followed by the Fe-C, GFF-C, and Am-C, respectively, indicating that the bioelectrochemical metabolic process for methane production depends on the electrochemical properties of the cathode surface.

Table 3.5 Summary of estimated bioelectrochemical methane production from different cathodes

Parameters	Stage	Ni-C	Fe-C	Am-C	GFF-C	Control
P_u (mL CH_4 /g COD)	E	215.9	163.0	166.0	158.4	114.2
	M	237.3±8.4	176.2±1.9	178.9±6.7	156.8±10.0	89.2±16.6
μ_m(mLCH_4/ g COD.d)	E	6.2	5.4	3.6	3.7	2.4
	M	44.8±2.4	35.4±1.6	34.0±1.9	28.5±2.3	17.8±3.8
λ (d)	E	22.51	20.49	9.38	13.01	6.90
	M	0.35±0.15	0.65±0.12	0.89±0.20	1.38±0.34	1.53±0.28
r^2	E	0.98	0.99	0.98	0.99	0.97
	M	0.98±0.00	0.987±0.01	0.99±0.00	0.99±0.01	0.99±0.00
Yield (mL CH_4/g COD_r)	M	326.3±13.9	235.9±17.9	272.2±20.4	252.8±12.5	162.1±18.9

(E: EAB enrichment stage, M: matured stage after the EAB enrichment)

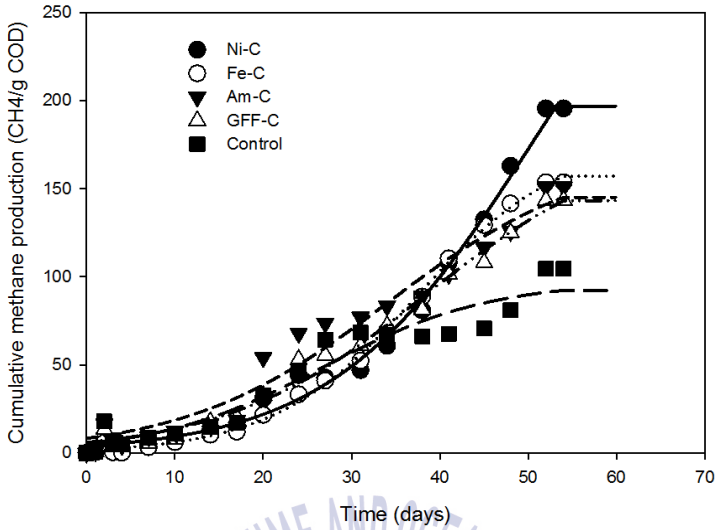


Fig. 3.7. Cumulative methane productions from the bioelectrochemical reactors with the cathodes decorated with different materials during the enrichment stage of EAB.

③ Bioelectrochemical methane productions from different cathodes

After the enrichment of EAB on the cathode surface, the influence of the decoration materials on bioelectrochemical methane production were distinct (Fig. 3.8). The cumulative methane production was quickly increased after a short lag phase when the substrate medium was replaced with a fresh one (Table 3.5). The maximum methane production rate is an indicator of the methanogenic potential of EAB on the cathode, which was obtained from the greatest slope of the cumulative methane production. The maximum methane production rate was 28.5 mL CH_4/g COD.d for GFF-C, which was higher than the value of 17.8 mL CH_4/g COD.d for the anaerobic control (Table 3.5). This shows that the bioelectrochemical activity for methane production is higher than conventional anaerobic digestion. However, the maximum methane production rate for the Ni-C was considerably higher, namely 44.8 mL CH_4/g COD.d. This result suggests that the bioelectrochemical methanogenic activity

was significantlyimproved by the decoration of Ni and MWCNT on the cathode surface. The maximum methane production rates for the Fe-C and the Am-C were similar to one another, but were less than the Ni-C. It is believed that the bioelectrochemical methanogenic activity is a function of EAB species and their biomass on the cathode surface. The species and biomass of EAB are possibly affected by the decoration materials assisting the electron transfer of EAB on the cathode surface (Cercado et al., 2013).

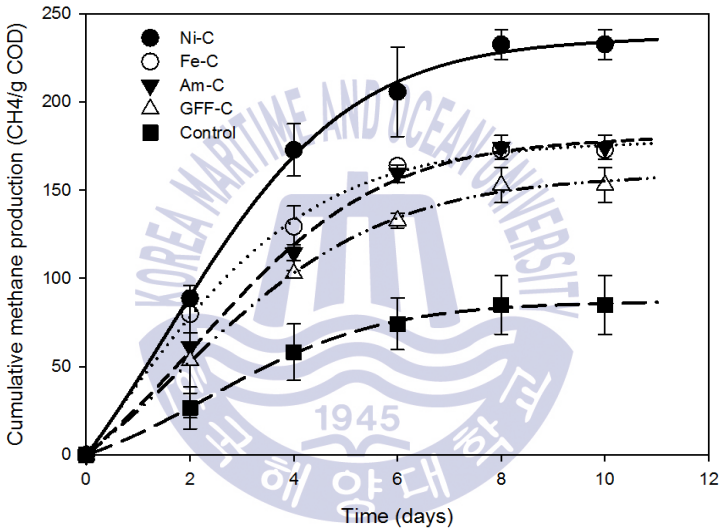


Fig. 3.8. Cumulative methane productions from the bioelectrochemical reactors with the cathodes decorated with different materials after the enrichment of EAB.

The bioelectrochemical methane yield was 326.3 mL CH_4/g COD_r for the Ni-C, which was 29.1% higher than 252.8 mL CH_4/g CODfor the GFF-C (Table 3.4). However, the methane yields were 235 mL CH_4/g COD_r and 272 mL CH_4/g COD for both of the Fe-C and the Am-C, respectively, which are also higher than 162.1 mL CH_4/g COD for the anaerobic control. The overpotential for bioelectrochemical methane production leads to energy loss in the electrode reaction. The ultimate methane production of the Ni-C is

higher than the others, indicating that the energy loss of the Ni-C for the bioelectrochemical conversion of carbon dioxide into methane is less than the others. The applied voltage is distributed to electrodes (anode and cathode) depending on the electrochemical properties of the electrodes during the enrichment stage of EAB. However, the electrode potentials could be considerably changed by the enrichment of EAB on the electrodes. It seems that the electron balance on the anode and cathode determine the distribution of applied voltage to the electrodes (Feng et al., 2016a; Feng & Song, 2016a; Song et al., 2016).

④ Electrochemical properties of the decorated cathodes

The results of the EIS experiments for the decorated cathodes are fit well by a Randles equivalent circuit model in the Nyquist representation (Fig. 3.9a). This suggests that the reduction of carbon dioxide into methane was mainly controlled by charge transfer, and partly by diffusion on the cathode surface. However, the semi-circles were a little flat, and the centers were below the real axis. The incomplete flat semi-circle is commonly observed as the current is not uniform on the cathode due to the inhomogeneous electrode surface (Cheng and chen, 2013; Quintero et al., 2013). The estimated values of charge transfer resistance (Rct), capacitance (C),Warburg (W)for the cathodes decorated with the electron transfer assisting materials were presented in Table 3.6. The solution ohmic resistances for the cathodes were in the range of 1.24-1.33 ohms, which was similar to the GFF-C (1.27 ohms). The semi-circle diameter of the Nyquist plots is the charge transfer resistance. The charge transfer resistances were 3.1 ohm for the Ni-C, which was lower than those for the Fe-C or the Am-C. The charge transfer resistance for the GFF-C was 8.6 ohms. The activation energy is described by a function of the charge transfer resistance on the cathode surface using the Arrhenius equation (Yamada et al., 2010; Ishihara et al., 2014). The small charge transfer resistance suggests that the cathode has a kinetic advantage for bioelectrochemical methane production, indicating that Ni is a

better decoration material assisting the electron transfer of EAB on the cathode for the bioelectrochemical conversion of carbon dioxide into methane (Feng & Song, 2016a). The Tafel plot for the cathodes decorated with different materials provides more information regarding the methane production from the reduction of carbon dioxide. Commonly, a smaller activation energy for an electrochemical reaction is obtained from an electrode with a smaller Tafel slope and higher exchange current (Feng & Song, 2016a; Feng et al., 2016b). The Tafel slope for the Ni-C cathode was the smallest at-322.6 mV dec-1, followed by Fe-C (-362.6mVdec-1) and Am-C (-409.1 mVdec-1) (Table 3.6).The Tafel slope of GFF-C was the greatest as -474.2 mVdec-1, which was higher than those of the cathodes decorated with electron transfer assisting materials. For the exchange current, the Ni-C also obtained the greatest value of 35.10 mA, which indicates Ni is a better decoration material for bioelectrochemical methane production by assisting the electron transfer of EAB. The exchange current of Fe-C is 31.44 mA, which is similarto the29.03 mA of Am-C, and the smallest of GFF-C is 23.06 mA. This indicates that the bioelectrochemical conversion of carbon dioxide into methane is catalyzed by Ni, Fe, and ammonia and the GFF decorated with Ni and MWCNT is the best cathode for the bioelectrochemical production of methane.

Table 3.6 EIS data for different cathodes

Parameters	Ni-C	Fe-C	Am-C	GFF-C
Rs(Ω)	1.33	1.24	1.24	1.27
Rct(Ω)	3.10	5.60	5.56	8.60
C(mF)	59.4	32.5	29.2	18.1
W $(1/\Omega\sqrt{Hz})$	0.223	0.356	0.406	0.482
β_c (mV/dec)	-322.6	-362.6	-409.1	-474.2
i_0 (mA)	35.10	31.44	29.03	23.06

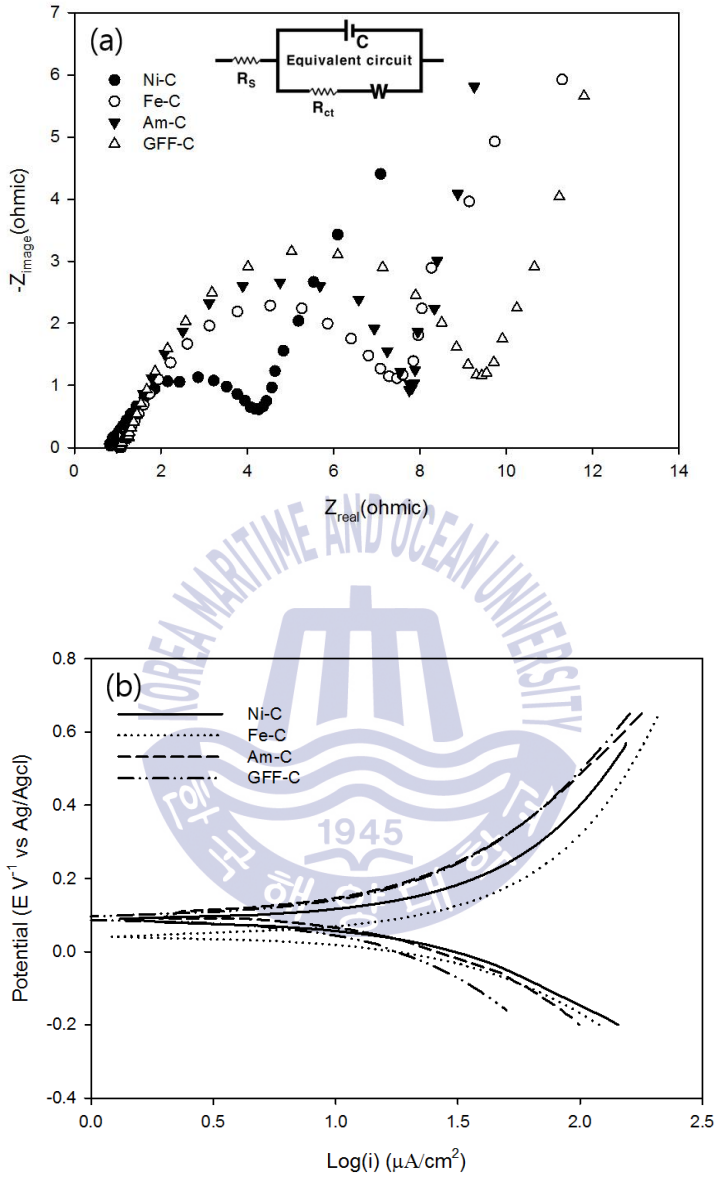


Fig. 3.9. (a) Nyquist plot of EIS data, b) Tafel plot for the cathodes decorated with different materials.

3.4 Conclusions

The surface modification of GFF anode affects on the bioelectrochemical methane production. The electrophoretic deposition of MWCNT and Ni on the GFF surface is a good method to improve the electric conductivity and electrochemical properties of the GFF. The scaffold layer of EG and MWCNT formed on the GFF surface provides a favorable structure for bacterial growth. The GFF anode modified with scaffold layer of EG and MWCNT using coal tar pitch binder after the electrophoretic deposition has smaller charge transfer resistance, compared to the control, and increases in the ultimate methane production and maximum methane production rate in bioelectrochemical anaerobic digestion. Coal tar pitch is a biocompatible binder for the enrichment of EAB and nickel is a good catalyst for electron transfer on the anode.

For the cathode, the GFF surface is well decorated with an electron transfer assisting material (Ni, Fe, or ammonia) together with MWCNT by the EPD method. The MWCNT loaded on the graphite fiber acts as an electrical bridge between graphite fibers. The decorations of MWCNT together with Ni, Fe, and ammonia considerably improve electrochemical properties of the GFF cathode. However, the decorated cathode with improved electrochemical properties requires a longer lag time for enrichment of EAB on the surface. Ni loaded on the GFF cathode considerably reduces the charge transfer resistance for the bioelectrochemical reduction of carbon dioxide on the cathode, followed by the Fe loaded GFF cathode, and finally, the ammonia. The decoration of MWCNT together with Ni on the cathode significantly improves the bioelectrochemical production of methane, as well as, the methane yield. The benefits of this material were then followed by the decoration of Fe and ammonia.

Chapter 4: Influence of HRT, Temperature and Applied Voltage on Bioelectrochemical Anaerobic Digestion for Sewage Sludge Treatment

4.1 Introduction

Anaerobic digestion is a traditional appropriate technology which has been used for over 100 years for the stabilization of organic waste and energy recovery in the form of methane. However, some concerns remain that need to be resolved, such as low organic matter degradation (e.g. <50% for sewage sludge), low methane content (<65%) in biogas, and process instability, all of which are mainly caused by slow rates of methanogenesis and hydrolysis, as well as the imbalance between acidogenesis and methanogenesis (Song et al., 2004). The slow methanogenesis rate and the imbalance of anaerobic reactions are mainly attributed to the slow growth rate of methanogens and their sensitivity to environmental changes, such as pH and temperature, or exposure to toxic compounds (Karakashev et al., 2005; Chen et al., 2008; Abu-Orf et al., 2014). The slow hydrolysis rate of particulate organic matter is often considered to be a rate limiting step in the overall anaerobic digestion of organic waste (Shin & Song, 1995; Luo et al., 2012a). Therefore, the general requirements for anaerobic digester operation are a long HRT (>20days), stable temperature (at 35 ℃ or 55 ℃) and controlled digester operation by a skilled hand1. Recently, several pretreatment attempts have been made using heat, ultrasound, and acid or alkaline to increase the hydrolysis rate (Zhang et al., 2010; Ariunbaatar et al., 2014). The increase in methanogenesis rate has also been attempted by increasing the biomass retention in the digester, however, only limited success was achieved (Bolzonella et al., 2005; Wang et al., 2011). Therefore, the bioelectrochemical technique could be a good available alternative to improve anaerobic digestion performance. A typical electrochemical reaction involves

the electron transfer between an electrode and reactants in a solution, and the reaction kinetic depends on the electrode potential. Bioelectrochemical reactions use microorganisms growing on the electrode surface as a biocatalyst. The electrode reactions in bioelectrochemical anaerobic digestion are described by the anode reaction: $CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$, E_{pa}=-0.486V (vs. Ag/AgCl), and the cathode reaction: $HCO_3^- + 9H^+ + 8e^- \rightarrow CH_4 + 3H_2O$, E_{pc}=-0.445V (vs. Ag/AgCl) under a biologically relevant condition at a pH 7.011. From the relationship ($\triangle G = -nFE$) between electrode potential and free energy change, the driving force for the anodic oxidation becomes theoretically larger at a more positive electrode potential than Epa and the cathodic reduction, becomes theoretically larger at a lower, more negative than Epc. The bioelectrochemical methane production from the reduction of proton and carbon dioxide was confirmed at −0.65V (vs. Ag/AgCl) of the cathode potential, and significantly increased at more negative cathode potentials (Cheng et al., 2009; Villano et al., 2010; Sasaki et al., 2011; Sasaki et al., 2013). Recently, enhanced methane production was shown to be possible in a bioelectrochemical reactor with a low applied voltage of 0.3 V, but higher applied voltage led to more accumulation of hydrogen (Wang et al., 2009; Feng et al., 2015). The optimal electrode potentials for oxidation and reduction in a bioelectrochemical anaerobic digester are still ambiguous. The bioelectrochemical methane production is certainly influenced by the design parameters including electrode materials, electrode size, and arrangement in the digester, as well as the operational parameters including HRT, temperature, and applied voltage. However, the studies on the design and operational parameters for the bioelectrochemical methane production are still limited.

In the present study, a lab scale anaerobic bioelectrochemical digester for sewage sludge was studied at different HRT, temperature and applied voltage. The performance of the digester including digester stability, organic matter removal, biogas production, and methane content in the biogas were investigated. The results of HRT and applied voltage experiments were

published at 'Energy and Fuels' and 'Bioresource Technology', respectively (Song et al., 2016; Feng et al., 2016a).

4.2 Materials and Methods

4.2.1 Preparation of anode and cathode

The surface of graphite fiber fabric (GFF, Samjung C&G Co., South Korea) was modified with a multiwall carbon nanotube (MWCNT, Carbon Nano-material Technology Co., Ltd., South Korea) and nickel (Sigma-Aldrich Co., USA) by using an electrophoretic deposition (EPD) method (Kaya et al., 2005; Park et al., 2008), and was used as the cathode. In short, the pristine GFF and MWCNT were submerged in concentrated nitric acid for 24 hrs to increase the hydrophilicity of the surface. An electrolyte solution was prepared by dissolving 1g MWCNT, 0.25g nickel chloride, and 0.5g polyethylenimine in 1L distilled water. The GFF was submerged in the electrolyte solution, and the MWCNT and nickel were simultaneously deposited on the surface of the GFF by an applied voltage of 30V for 30min using a DC (direct current) power source (OPM series, ODA Technologies Co. Ltd, Incheon, South Korea). The GFF was used as a working electrode while a stainless steel mesh was used as the counter electrode for the modification of the GFF surface. An anode was prepared by a screen printing with a mixture paste of the MWCNT and exfoliated graphite (EG, Hyundai Coma Industry, Inc., Seoul, South Korea) on the surface of the modified GFF. A mixture of Nafion solution (50% wt., Sigma-Aldrich Co., St. Louis, USA) and epoxy (50% wt.) was used as a binder for the screen printing on the electrode. The separator and electrode assembly (SEA, 6cm×24cm) was prepared by stacking the anode, a polypropylene non-woven sheet as a separator, and the cathode in that order.

4.2.2 Bioelectrochemical anaerobic digester

A cylindrical anaerobic digester (diameter 24cm, effective volume 12L) was

prepared with acrylic resin, and six sets of the SEAs were helically installed inside of the digester (Fig. 4.1). Each electrode (anode, cathode) of the SEAs was connected to a DC power supply (OPM series, ODA Technologies Co. Ltd, Incheon, South Korea) using a conductive wire. The anaerobic digester was covered with an acrylic resin plate for sealing, and a water replacement type gas collector for biogas monitoring was connected to a port on the cover plate using a rubber tube. The biogas collector was filled with the water which was acidified with sulfuric acid and saturated with salt to prevent the resolution of the biogas. A biogas sampling port capped with a butyl rubber stopper was installed on the cover plate. For the start-up of the digester, the potential difference between the anode and the cathode was set to 0.3 V using the DC power supply, and around 40% of the effective digester volume was filled with seed sludge which was taken from a sewage sludge anaerobic digester (Busan, South Korea). The digester content was completely mixed with a blade using a motor (100rpm), and the digester temperature was controlled to 35 ℃ using a water bath. Sewage sludge, a mixture of primary sludge (ca. 25%) and waste activated sludge, was obtained from a thickening tank of the Y sewage treatment plant (Busan, South Korea), and fed into the bioelectrochemical digester once a day. The characteristics of the seed sludge and feed sewage sludge are listed in Table 4.1.

Table 4.1 Characteristics of the seed sludge and feed sewage sludge

Parameters	Seed sludge	Feed sewage sludge
pH	6.34	6.0±0.17
Alkalinity (mg/L as $CaCO_3$)	582	1,132.2±340.5
TVFA (mg/L as HAc)	108	520.6±142.9
TS (g/L)	15.6	41.6±8.0
VS (g/L)	10.6	28.8±5.2
TCOD (g/L)	18.4	36.6±0.5
SCOD (g/L)	2.7	1.8±0.8

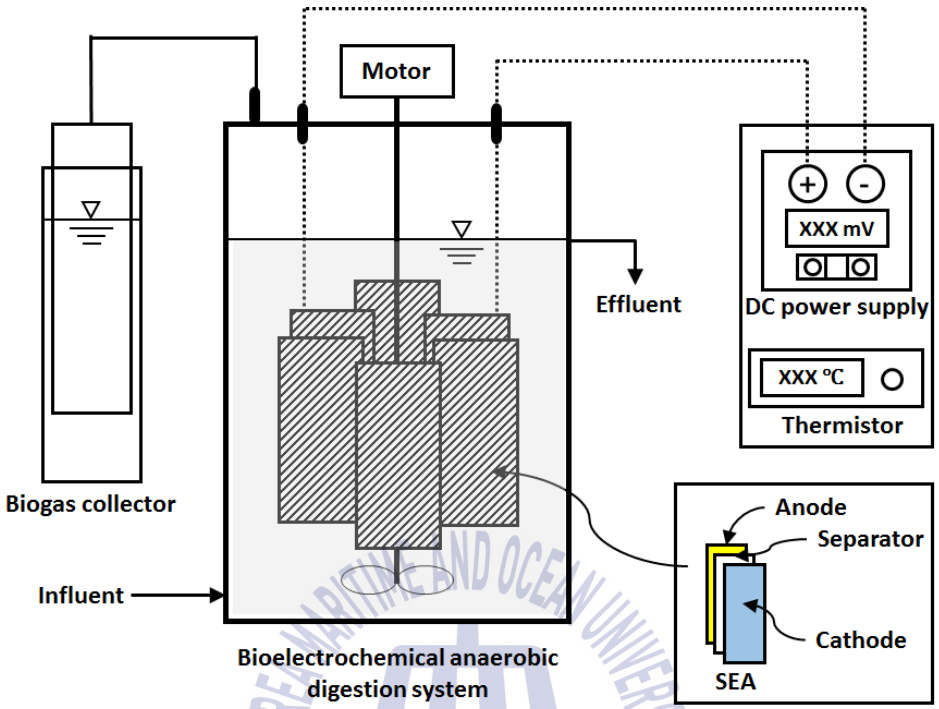


Fig. 4.1. Schematic diagram of bioelectrochemical anaerobic digester and SEA.

4.2.3 HRT for bioelectrochemical anaerobic digestion

The HRT for the bioelectrochemical anaerobic digester was varied with 20 days, 15 days, 10 days and 5 days by a step increasing of the sewage sludge feeding rate. At each HRT condition, the bioelectrochemical anaerobic digester was operated for the period more than three times of the HRT, and the steady state was confirmed from the stability of some state variables, such as pH, alkalinity, methane production, methane content in biogas, and organic matter removal.

4.2.4 Temperature for bioelectrochemical anaerobic digestion

The bioelectrochemical anaerobic digester was started up at 35 ℃ with 10 days of HRT, and then the temperature was downshifted to 25 ℃ from the 33rd day when the state variables and performance was stabilized. In the 71st

day, the HRT was increased to 20 days without a change of temperature condition in the bioelectrochemical digester.

4.2.5 Applied voltage for bioelectrochemical anaerobic digestion

The bioelectrochemical anaerobic digester was inoculated with the electroactive sludge which was taken from another mesophilic bioelectrochemical anaerobic digester. The HRT was set to 20 days during the whole experimental period. The bioelectrochemical digester was started by setting the applied voltage to 0.5V between the anode and cathode, and mainted at ambient temperature (25±2 ℃). The applied voltage between the anode and cathode was adjusted from 0.5V to 0.7V as the state variables were stabilized, and then to 0.3V.

4.2.6 Analysis and calculation

During operation of the bioelectrochemical anaerobic digester, the pH and alkalinity for the feed sludge and the digester effluent were monitored daily using a pH meter (Orion Model 370) and a titration method, respectively. The other parameters including total solids (TS), volatile solids (VS), total and soluble chemical oxygen demand (COD) were monitored twice a week according to Standard Methods (1995). The total VFA was measured using the titration method proposed by Anderson et al. (1992) (Anderson & Yang, 1992). Briefly, the sample is titrated to pH 5.1 and 3.5 by using 0.1N sulfuric acid, consecutively, and then the total VFA was calculated from the volume of consumed sulfuric acid and the volume of sample taken. The total VFA was used for the calculation of the ratio of VFA to alkalinity. Biogas production was monitored and converted to the standard temperature and pressure by the correction of water vapor pressure at 25 ℃, and the biogas composition was determined by a gas chromatography (Series 580, Gaw-Mac Instrument Co., PA, USA) with a thermal conductivity detector and Porapak-Q column. At a steady state, VFA composition and the level in the digester effluent were analyzed using a high performance liquid

chromatography (DX-500), with an Aminex HPX-87 column (300×7.8mm) employing ultraviolet detection. The potentials of the anode and cathode versus an Ag/AgCl reference electrode (RE-1B, ALS Co., LTd, Japan) were intermittently measured by using a portable digital multimeter (DM-1010, Dong Hwa Electronics, Co., South Korea). The current of the external circuit was monitored using a digital multimeter (DMM, Ni cDAQ-9174, National Instruments), installed between the electrodes and the DC power source. The overall energy efficiency of the energy recovered as methane relative to both electric energy input and substrate removed was estimated as the flowwing Equation 4.1 (Villano et al., 2010; Rader & Logan, 2010),

Equation 4.1: $\eta(\%) = \frac{W_{CH_4}}{W_s + W_E} \times 100$

where, $W_{CH_4}(= \triangle G_{CH_4} \times n_{CH_4})$ is the theoretical free energy change for the oxidation of methane into water and carbon dioxide ($\triangle G_{CH_4}$=-181kJ/mol) , and the methane production ($=n_{CH_4}$, moL.day) is the value at steady state. W_s(= $\triangle G_S \times n_s$) is the energy content estimated from the substrate removed, where $\triangle G_S$ (=-2,870kJ/mol) is the free energy change for the oxidation of glucose into water and carbon dioxide , n_s is the number of substrate moles removed, W_s(=$Q \times E_{app}$) is the supplied electric energy amount, Q is the total coulomb that was obtained by integrating the current by time, and is the potential difference between the anode and the cathode.

4.2.7 Pyrosequencing for microbial community analysis

The suspended sludge in the bioelectrochemical digester was taken at steady state of each applied voltage, and the microbial community profile of the sludge was analyzed by a pyrosequencing based on the 16S rRNA gene. The 16S rRNA gene was amplified from metagenomic DNA using a 454 GS FLX Junior Sequencing System (Accession number CP003117, Roche, Branford, CT, USA). In accordance with previous studies (Chun et al., 2010), amplification, construction of the sequencing library, sequencing and bioinformatic analyses were performed. The samples for high quality

sequences were allocated by bar code. For the raw sequence read, samples were separated from their origins, and any reads less than 25bp and higher than 300bp were removed, and 200bp of the minimum length was selected. The sequence was aligned using the SILVA alignment tool (Pruesse et al., 2007) and clustered into operational taxonomic units (OTUs). The OTUs were selected after reaching a variation level of 0.10, and the microbial community and statistical taxonomical assignments were obtained through these OTUs. To cluster analysis and standardize the different reads used with the CL community software (Chunlab, Inc., South Korea), the Mothur program was used when exhibiting an 80% confidence level and 97% similarity with SILVA Random Calculation (Subha et al., 2015).

4.3 Results and discussion

4.3.1 Influence of HRT on bioelectrochemical anaerobic digestion

① Stability of bioelectrochemical anaerobic digester (pH, alkalinity and VFA)

An anaerobic digester becomes easily stressed by excess organic loading rate or severe variations of the digester input. VFAs can then be accumulated in the digester due to the imbalance between the VFAs production from organic matter and the conversion to methane. The accumulation of the VFAs leads to a pH drop, and eventually the digester deteriorates (Padilla-Gasca et al., 2011). Therefore, anaerobic digester stability can be defined as the ability to withstand the capacity of the fluctuations of the digester input, and it is commonly a matter of great concern for successful anaerobic digester operation (Komemoto et al., 2009). The digester input is the physico-chemical properties of the feed sewage sludge such as pH, alkalinity and substrate, as well as the operational conditions such as HRT, organic loading rate, and temperature.

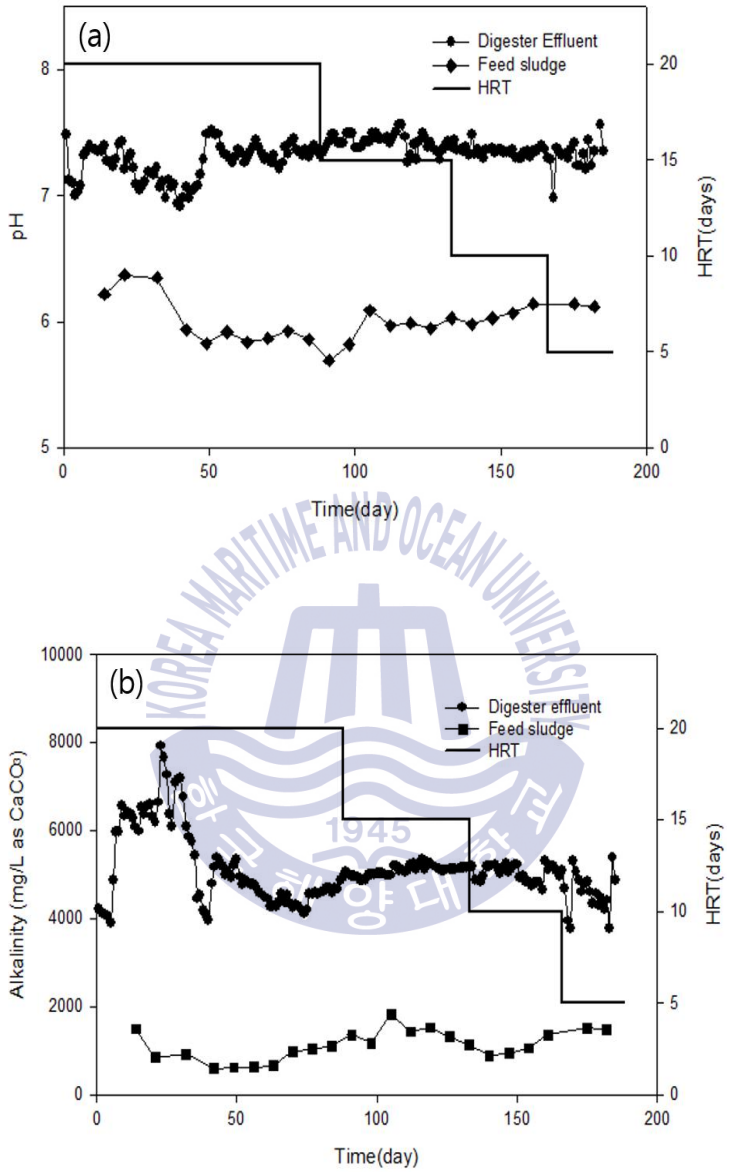


Fig. 4.2. Changes of pH (a) and alkalinity (b) for the bioelectrochemical anaerobic digester during the operation.

When the bioelectrochemical anaerobic digester was operated at 20 to 5 days of HRTs, the organic loading rates (OLRs) were in the range of 1.44 to 5.76 g VS/L.d, and the variability of pH (5.7-6.4) and alkalinity (600-1,830mg/L as $CaCO_3$) of the feed sewage sludge were considerable (Fig. 4.2). Anaerobic digester stability could be evaluated from the variability for both the state variables of the digester (such as pH, VFAs, alkalinity, organic matter removal, and biogas production), and their deviations from their optimum values for anaerobic digestion.

After the start-up period (about 50days) of the bioelectrochemical anaerobic digester, the pH was quite stable at 7.2-7.6, which was favorable for anaerobic bacterial growth, during the entire operation time (Fig. 4.2a). When the HRT was changed from 10 to 5 days, the pH temporarily reduced to 6.9, but quickly recovered to 7.4 in the following day. In the HRT range of 20 to 10 days, pH change due to the abrupt step change of HRT was not observed. Generally, pH in the anaerobic digester is determined by a combination of some state variables including alkalinity, carbon dioxide in biogas, and the VFA, which are affected by digester input parameters such as the characteristics of feed sludge and operational conditions (Song et al., 2004; Demitry & McFarland, 2015). In the bioelectrochemical digester, the alkalinity was stable at 4,860 mg/L (3,780-5,450) as $CaCO_3$ (Fig. 4.2b). The alkalinity was not affected by the HRTs ranging from 20 to 5 days, and the temporary drop due to the step change of HRT from 10 to 5 days was quickly recovered, similarly to the pH. In a previous study, the alkalinity in the conventional anaerobic digester for sewage sludge was around 4,000-6,400mg/L as $CaCO_3$, which was higher than the value in the bioelectrochemical digester (Song et al., 2004; Kardos et al., 2011; Peces et al., 2013). The major components of alkalinity in the anaerobic digester are bicarbonate and carbonate, indicating that the relatively low alkalinity in the bioelectrochemical digester is attributed to the reduction of the carbon dioxide into methane. The total VFA/alkalinity ratio is also an indicator of the anaerobic digester stability. The ideal value of total VFA/alkalinity ratio is in

the range of 0.1 to 0.3, and values over 0.4 indicates an instable operation of anaerobic digester and usually involve VFAs accumulation (Rader & Logan, 2010). The total VFA/alkalinity ratio of the bioelectrochemical anaerobic digester was maintained at 0.12-0.23 during the entire operation time (Table 4.2). The above results show that the pH, alkalinity, and total VFA in the bioelectrochemical digester were in the normal range and were very stable during the entire operation at 20 to 5 days of HRTs, indicating that the bioelectrochemical anaerobic digester has a great capacity to withstand the fluctuations of the digester input parameter.

② VS and COD reduction

The VS of the feed sewage sludge was varied from 21,800 to 32,600mg/L during the entire operation time after the start-up time, but the effluent VS in the biochemical anaerobic digester was comparatively stable (12,000±3484 mg/L) (Fig. 4.3a), indicating that the VS reduction was mostly affected by the VS fluctuations of the feed sludge. At 20 days of HRT, the VS reduction was 70.5% (Table 4.3), which was an extremely high value compared to the conventional anaerobic digester (Song et al., 2004; Peces et al., 2013; Takashima et al., 2014). The main component of sewage sludge is particulate organic matter mainly composed of proteins and carbohydrates (Chen et al., 2007; Mottet et al., 2010). The anaerobic degradation of sewage sludge is commonly limited by the hydrolysis of the particulate matter. In a conventional anaerobic digester, the percentage of VS reduction for sewage sludge is around 30%-50%, which does not differ at over 15 days of HRT due to the limited readily degradable organic content in the sewage sludge (Song et al., 2004; Metacalf et al., 2014; Luo et al., 2012a; Peces et al., 2013; Takashima et al., 2014). It is considered that the lowest limit of HRT in a conventional anaerobic digester for sewage sludge is around 10 days. In the bioelectrochemical digester, the VS reduction gradually decreased with the decrease in HRTs ranging from 20 to 5 days, and it was 52.2% at 5 days of HRT (Table 4.3).

Generally, the anaerobic biodegradability of sewage sludge based on COD ranges from 33% to 75%, depending on the primary sludge content and the types of biological processes (Ikumi et al., 2014; Strauber et al., 2012; Corazza et al., 2005). In this study, the total COD removal was around 64.0% at 20 days of HRT (Fig. 4.3b). It is likely that almost all of the biodegradable fraction in the feed sludge was degraded in the bioelectrochemical anaerobic digester. The removal efficiency of the total COD was decreased to 43.5% at 15 days of HRT, similar trend with the changes of the VS reduction. The COD removal efficiencies were 40.3% at 5 days of HRT and 38.7% at 10 days of HRT. It is very important to discuss the cause of the high reduction of COD and VS in the bioelectrochemical digester. The particulate organic matter is hydrolyzed by some extracellular enzymes such as protease, amylase, and endo-glycanase (Luo et al., 2012a; Strauber et al., 2012). The hydrolysis reaction is governed by the enzyme concentration and reactivity in the anaerobic digester. The poor reactivity of the hydrolytic enzymes is ascribed to the uncompetitive or noncompetitive inhibition of the substrate, and the competitive inhibition of the hydrolysis product (Corazza et al., 2005). However, the substrate inhibition of hydrolysis reflects the intrinsic physico-chemical characteristics of the sewage sludge, possibly altered only by pretreatments using heat, acid or alkaline, and ultrasound. It is possible that the hydrolysis product is a more important factor determining the sewage sludge hydrolysis. The hydrolytic enzyme is produced by the acidogenic bacteria during the fermentation of the hydrolysis products into the range of products such as various volatile fatty acids (VFAs), alcohols, hydrogen, and carbon dioxide. The production of hydrolytic enzyme is inhibited by the hydrolysis products and the fermenting production. During the entire operation time, the SCOD in the bioelectrochemical digester was 1,100mg/L or less, which was considerably lower than that of 2,560mg/L in the conventional anaerobic digester at 20 days of HRT by using the sewage sludge in previous study (Table 4.2) (Song et al., 2004). This is possibly because the hydrolysis reaction in the bioelectrochemical digester was enhanced by the low SCOD, indicating the overall products for the hydrolysis

and the acidogenic fermentations. The changes of SCVFAs and their compositions at different HRTs are presented in Table 4.2. The SCVFA in feed sewage sludge varied from 2,830 to 4,270mg HAc/L during the digester operation. The SCVFA in the digester effluent was quite low and stable at over 10 days of HRTs at 338-409 mg COD/L, but it increased to 616±26 mg COD/L at 5 days of HRT (Table 4.2). In the anaerobic digester, the VFA increases with the decrease in HRT or the increase in organic loading rate if the methanogenic activity is insufficient for its control. It seems that the lowest limit of HRT in the bioelectrochemical anaerobic digester for sewage sludge is less than 5 days. The lower SCVFA level in the bioelectrochemical digester is closely linked to its higher bioelectrochemical methanogenic activity. It is believed that the enhanced hydrolysis reaction in the bioelectrochemical digester was also attributed to low SCVFA.

Table 4.2 Properties of bioelectrochemical anaerobic digester for sewage sludge at different HRTs

Variables\HRT (days)	Bioelectrochemical anaerobic digestion				Conventional AD
	20	15	10	5	20
OLR (kg VS/m3.d)	1.44	1.92	2.88	5.76	1.43, 1.2
pH	7.35±0.07	7.42±0.07	7.36±0.04	7.34±0.08	7.67, 7.2-8.0
Alkalinity (mg/L as $CaCO_3$)	4,541±250	5,088±114	5,048±177	4,619±451	6,412, 4,000-6,000
SCVFAs (mg COD/L)	408.9±52	337.9±25	344.5±34	615.5±26.2	618, 1,600, 33.2
HFo (%) : HAc (%) : HPr (%)	8.5:50.1:41.4	12.2:77.5:10.3	9.1:77.4:13.5	4.1:93.5:2.4	0:97.6:2.4 0:45.9:23.2
TVFA/Alkaliniy	0.16±0.03	0.15±0.03	0.18±0.02	0.18±0.05	0.10, 0.27-0.67
SCOD (mg/L)	1092±36	1068±208	439±130	749±36	2,555, 1,242-7,939
Potential (mV) Anode	-200±24	-236±15	-241±20	-249±17	-
Potential (mV) Cathode	-503±18	-536± 13	-542±25	-546±21	-
Current density (mA/L)	226.5±0.2	231.5±0.3	233.1±0.1	233.4±0.2	-

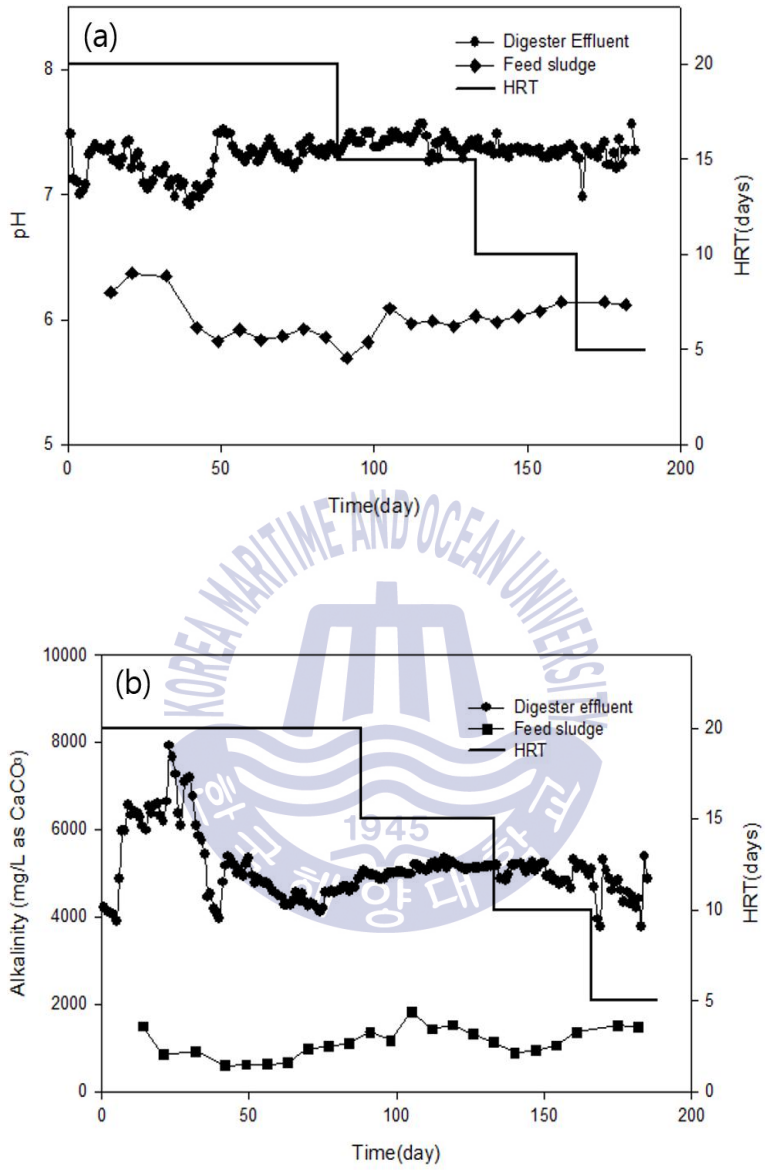


Fig. 4.3. (a) VS and (b) TCOD in feed sludge and digester effluent and their removals (%) at different HRTs.

The VFA composition in the anaerobic digester gives more information on the state of the anaerobic digester. During the operation of the bioelectrochemical anaerobic digester, the main components of SCVFA were formic acid, acetic acid, and propionic acid, and their composition was affected by the HRTs (Table 4.2). At 20 days of HRT, the SCVFAs were composed of formic acid (8.5%), acetic acid (50.1%), and propionic acid (41.4%). The formation of short chain fatty acids is thermodynamically favorable at low hydrogen partial pressure, indicating that hydrogen scavenging syntrophs are a dominant microbial group in the digester (Thiele et al., 1988; Shrestha et al., 2014). However, it was interesting that the propionic acid portion was considerably reduced, and the acetic acid was increased to 77.5% at 15 days of HRT. In a previous study, the optimum temperature for the growth of propionic acid producing bacteria such as Paludibacter Propionicigenes gen. nov., sp. nov. was 30℃, and its growth rate considerably decreased at 35 ℃ (Ueki et al., 2006). At 15 days of HRT, the smaller fraction of propionic acid in the VFA may be attributed to the washout of propionic acid producing bacteria. At 5 days of HRT, the VFA was mostly acetic acid (93.5%), and the propionic acid fraction was further decreased.

③ Methane production

The total biogas production in the bioelectrochemical anaerobic digester increased with the decrease in HRTs from 20 to 5 days, but the methane content in the biogas was very stable (Fig. 4.4). The specific methane production rate at 20 days of HRT was 407 mL CH_4/L.d in Table 4.3, which was remarkably higher than that from the conventional anaerobic digester for sewage sludge (70-370 mL CH_4/L.d) (Song et al., 2004). The specific methane production rate at shorter HRTs was higher due to the

increase in organic loading rate, and the maximum value of to 1,339 mL CH_4/L.d was obtained with 5 days of HRT. In the bioelectrochemical anaerobic digester, the methane is produced from the bioelectrochemical reaction through direct electron transfer on the electrodes, and the indirect electron transfer of planktonic methanogenic bacteria is another important source of methane (Zhao et al., 2015). The direct electron transfer reactions for methane production are described as the oxidation of VFAs at the anode and the reduction of carbon dioxide at the cathode (Hamelers et al., 2010; Zhao et al., 2015). At 20 days of HRT, the electrode potentials were −200±24mV vs. Ag/AgCl for the anode and -503±18mV vs. Ag/AgCl for the cathode (Fig. 4.5). The anode potential is more positive than Epa (-486mV vs. Ag/AgCl) and the cathode potential is more negative than Epc (-445mV vs. Ag/AgCl), indicating that the electrode potentials are favorable for the bioelectrochemical oxidation of acetate and the methane production on the anode and the cathode, respectively. In previous studies, the reduction rate of carbon dioxide to methane was negligible at cathode potentials less than −700mV vs. Ag/AgCl (Cheng et al., 2009; Villano et al., 2010). However, the actual potentials for the biochemical reactions on the electrodes are affected by the overpotentials of the system (Villano et al., 2010). In the present study, the high performance of the bioelectrochemical anaerobic digester could be attributed to the low system overpotentials, compared to the previous studies (Cheng et al., 2009; Villano et al., 2010). Meanwhile, electroactive microorganisms such as *Shewanella* and *Geobacter* species are capable of secreting electron shuttles such as riboflavin and flavin mononucleotide at a specific electrode potential (Yang et al., 2012; Wu et al., 2013). The electron shuttles accept the electrons from planktonic acidogenic bacteria and deliver them to the planktonic methanogenic bacteria in liquid or the attached methanogenic bacteria at the electrode. It is believed that the electron shuttles also play a key role in the enhancement of the methane production rate in the bioelectrochemical anaerobic digester.

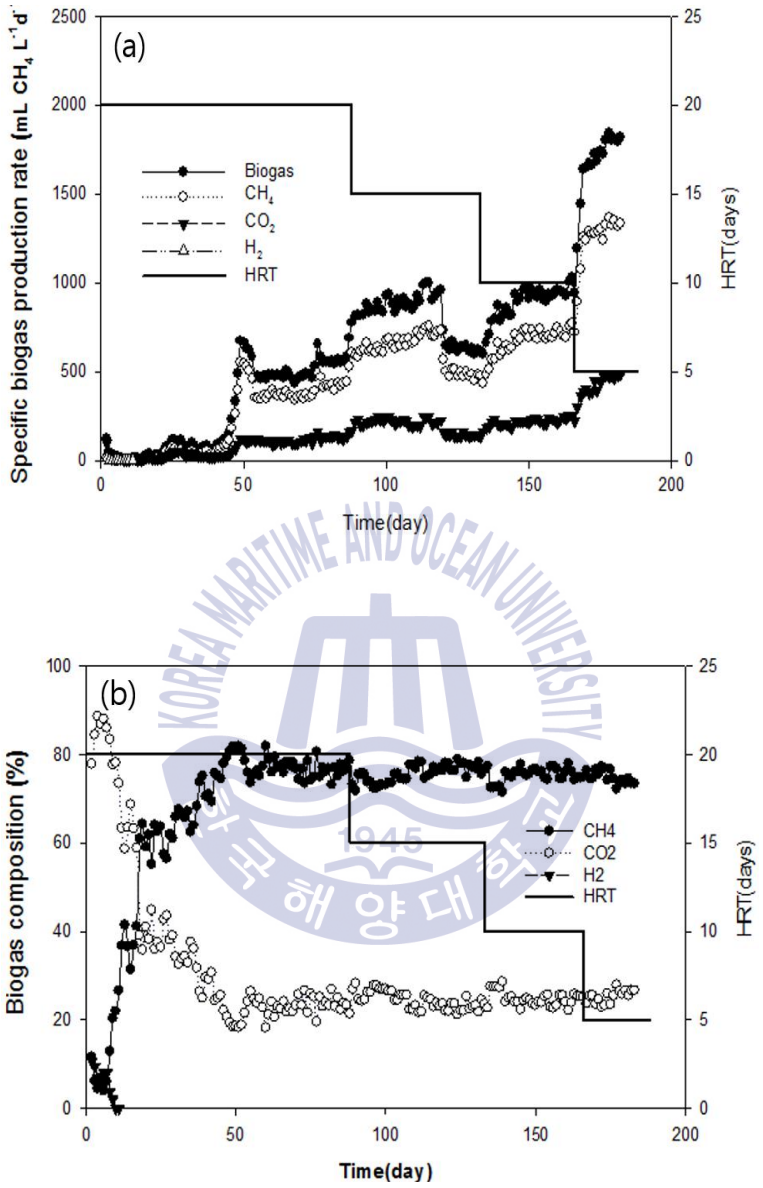


Fig. 4.4. (a) Specific biogas production rate and (b) biogas composition in bioelectrochemical anaerobic digester.

Table 4.3 Performance of bioelectrochemical anaerobic digester for sewage sludge at different HRTs

Contents		Bioelectrochemical anaerobic digestion				Conventional AD
HRT (days)		20	15	10	5	20, 20-21, 20
VS removal (%)		70.5±1.5	63.1±2.5	55.2±4.2	52.2±0.3	43.5, 36.9, 30-42
TCOD removal (%)		64.0±1.1	43.5±3.0	40.3±1.5	38.7±1.6	35.8, 31-46
SMPR (mL/L.d)		407±16	498±27	717±16	1339±19	203, 70-180, 370, 294-344
CH_4 (%)		76.9±0.7	77.2±0.8	75.2±0.9	73.5±0.8	64.7, 58.7-62.8
CH_4 yield (mL/g COD_r)	Total	368±7.6	430±12.3	479±11.7	414±14	123-263, 289.2-369.0
	PAB	207	266	314	248	-
	BEC	161	164	165	166	-
Energy efficiency (%)	Overall	69.1	90.5	98.7	89.4	-
	Net	58.6	87.1	98.4	88.1	-

PAB: planktonic anaerobic bacteria, BEC: bioelectrochemical conversion on electrode

At over 15 days of HRTs, the methane content in biogas was around 77%, as shown in Fig. 4.4b, which was significantly higher than that of the conventional mesophilic anaerobic digester (55%-65%) for sewage sludge (Song et al., 2004; Takashima et al., 2014). The methane content in biogas was slightly decreased to 75.2% and 73.5% at 10 and 5 days (Table 4.3), respectively, which were still higher than the conventional anaerobic digester. The biogas composition from the anaerobic digester can be stoichiometrically determined by the chemical composition and the biodegradability of the substrate. However, the carbon dioxide content in biogas is commonly lower than the theoretical value due to the higher solubility of the carbon dioxide in the digester content, indicating that the methane content in biogas is higher at higher pH in the anaerobic digester. During the entire operation time, the pH of the bioelectrochemical digester ranged from 7.34 to 7.42, which was

an ordinary range for an anaerobic digester. In the bioelectrochemical anaerobic digester, the methane contents (73.5%-77.2%) in biogas beyond the theoretical value could be ascribed to the additional bioelectrochemical conversion of carbon dioxide to methane (Cheng et al., 2009; Villano et al., 2010; Sato et al., 2013).

The theoretical methane yield of sewage sludge can be calculated 350 mL CH_4/g COD_r from the Buswell equation, but the observed methane yield in anaerobic biodegradability test was 123-352 mL CH_4/g COD_r in previous studies (Table 4.3) (Takashima et al., 2014; Astals et al., 2013). The methane yield of the bioelectrochemical digester was 368-479 mL CH_4/g COD removed, which is significantly higher than the theoretical values. It is well known that the bioelectrochemical reaction directly relates to the electrons transferred on electrode (Cheng et al., 2009; Villano et al., 2010; Sasaki et al., 2011; Sasaki et al., 2013), and the methane amount produced from the bioelectrochemical conversion on the electrode can be theoretically estimated from the electric energy input. This indicates that the high methane yield of the bioelectrochemical digester is partly ascribed to the methane from the bioelectrochemical conversion of carbon dioxide. Then, when HRT was decreased from 20 days to 5 days, the changes of electric current density were very small in this study (Table 4.2), but the fraction of methane yield from the planktonic anaerobic bacteria was considerably increased (Table 4.3). It suggests that the methane conversion efficiency of the planktonic bacteria through the indirect electron transfer is also improved by the role of electron shuttle, and the secretion of electron shuttle from electroactive bacteria on the electrode is affected by the organic loading rate.

The potentials of anode and cathode shifted to more negative values (−249±17 and −546±21mV vs. Ag/AgCl) as the HRT was varied from 20 days to 5 days (Fig. 4.5). The absolute potentials of the anode and cathode in a bioelectrochemical system are determined by the rate difference between the oxidation on the anode and the reduction on the cathode. The shift of electrode potentials in a negative direction indicates that the oxidation rate is

higher than the reduction rate. This means that the cathodic reduction of carbon dioxide to methane was a limiting step in the electrode reactions at shorter HRTs.

The overall energy efficiency for methane recovery relative to both electric and substrate energy inputs was varied from 69.1% to 98.7% at the HRT ranged from 20 to 5 days, and the maximum value was obtained at 10 days of HRT (Table 4.3). Then, the electric energy input was 70.5-72.6 kJ, and the considerable increase was not observed at longer HRT (Fig. 4.6). However, the fraction of the electric energy to the energy recovered as methane was 36.8% at 20days of HRT, and it was decreased to 12.6% as the HRT was decreased to 5days. The net energy efficiency based the energy recovered as methane except for the electric energy input was also affected by the HRT and the maximum value was also obtained at 10 days of HRT. This indicates that the methane portion produced from planktonic anaerobic bacteria increases at higher organic loading rate, contrary to the steady portion of the methane from the electroactive bacteria on the electrode.

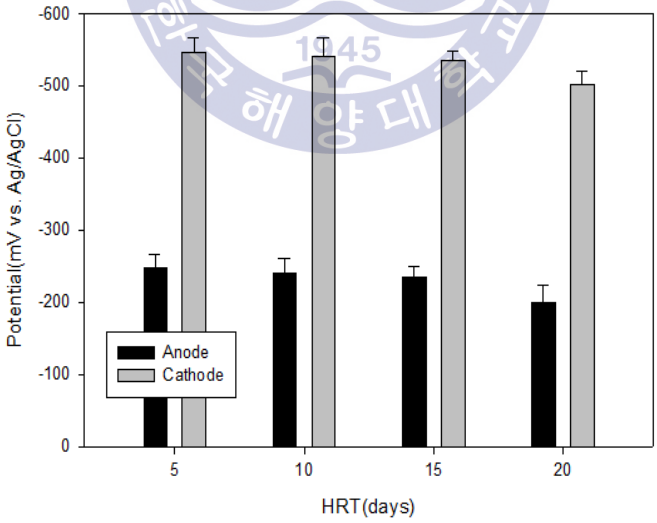


Fig. 4.5. Changes of electrode potentials with different HRTs in bioelectrochemical anaerobic digester for sewage sludge.

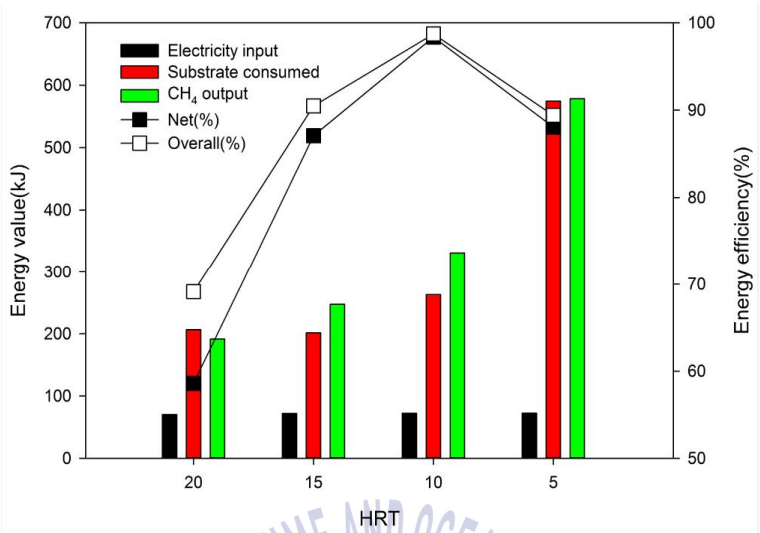


Fig. 4.6. Energy efficiency of bioelectrochemical anaerobic digester and energy values of substrate, electricity, and methane at different HRTs.

④ Implications in design and application

The state variables in bioelectrochemical anaerobic digester using small electric energy are very stable, and the process performance in organic removal, methane production and biogas purity is considerably enhanced. The bioelectrochemical anaerobic digester is easily constructed by installing anode and cathode inside the existing conventional anaerobic digester and it is operated by maintaining the potentials of electrodes. Some initial capital cost would be necessary for the electrode installation and the purchase of the DC power supply, but the profits in the improved performance will quickly compensate the cost during the operation of the bioelectrochemical anaerobic digester (Liu et al., 2012). The performance of the bioelectrochemical anaerobic digester depends on the electrode material, size, shape and the configuration (Sasaki et al., 2013; Rader & Logan, 2010; Zhao et al., 2015). Specially, the performance is affected by the interaction between planktonic anaerobic bacteria and electroactive bacteria on electrodes36. Some operational

parameters such as electrode potentials, HRT, organic loading rate, and temperature have significant influences on the microbial interaction (Bolzonella et al., 2005; Hamelers et al., 2010; Wirth et al., 2015). The bioelectrochemical anaerobic digester was very stable in the HRT ranged from 20 days to 5days. However, the performance was varied depending on the different HRTs. The methane content in biogas and organic removal efficiency was the highest at the longer HRT of 20days. However, the methane yield and energy efficiency were the best at 10days of HRT. The specific methane production rate was higher at higher organic loading rate. The recommended HRT varies in different purposes of the digester operation. The optimized electrode and operational conditions in the near future will increase the operational profits of the bioelectrochemical anaerobic digester.

4.3.2 Influence of temperature on bioelectrochemical anaerobic digestion

① Methane production in bioelectrochemical anaerobic digester

The methane production from the bioelectrochemical anaerobic digester was rapidly increased after start-up at 35 ℃, and then it was stabilized from the 14th day (Fig. 4.7a). This rapid start-up of the bioelectrochemical digester is possibly attributed to the EAB dominating inoculum, which was obtained from an old bioelectrochemical digester. The easy adaptation of EAB to the new environment is a valuable property in field application of the bioelectrochemical anaerobic digester. Generally, it takes a long time to get stable methane production for a bioelectrochemical anaerobic digester using conventional anaerobic digester sludge as the inoculum (Song et al., 2016). In a bioelectrochemical digester, the rapid adaptive increase in methane production is similar to the immediate increase in voltage after resuming substrate supply in starved microbial fuel cell in a way (Song et al., 2015a; Song et al., 2015b). At steady state, the specific methane production rate and methane yield obtained from the bioelectrochemical digester were considerably high at 698.6 mL/L.d and 431.6 mL/g COD_r, respectively (Table 4.4). In the

conventional mesophilic anaerobic digester for sewage sludge, the specific methane production rate was only 180-280 mL/L.d at 20 days of HRT or longer in previous studies (Song et al., 2004; Bolzonella et al., 2005; Takashima et al., 2014). The main sources of methane production in the bioelectrochemical digester are suggested as: i) bioelectrochemical conversion of carbon dioxide into methane on the cathode, and ii) methanogenesis from planktonic methanogenic bacteria (PMB) (Villano et al., 2010). It seems that the high methane production in the bioelectrochemical digester is due to bioelectrochemical conversion of carbon dioxide into methane in addition to methane produced from PMB. The methane content in the biogas from the bioelectrochemical digester was 76.1%, which was quite stable from start-up (Fig. 4.7b). In the conventional anaerobic digestion for sewage sludge, the methane content in biogas is around 55-65% (Song et al., 2004; Takashima et al., 2014). The methane content in biogas produced from the PMB is probably similar to that of the conventional anaerobic digestion. However, it is believed that the biogas produced from bioelectrochemical conversion on the cathode contains mostly methane. This indicates that both the methane content in biogas and the methane yield could be significantly increased by the contribution of bioelectrochemical conversion into methane.

On the 33rd day, the methane production rate was immediately dropped by downshifting the temperature from 35 ℃ to 25 ℃. However, the methane production rate was recovered to a stable value within 10 days after the temperature change. Commonly, as a response to a rapid temperature drop, microorganisms change physiological properties to ensure surviving in the new environment (Barria et al., 2013; van Gestel et al., 2013). The immediate drop in methane production exposed to the low temperature is mainly due to the decrease in the enzymatic reaction rates (van Gestel et al., 2013). However, the recovery process is described by rapid adaptation through the induction of a set of specific proteins that help to tune cell metabolism and readjust it to the new temperature condition (Barria et al., 2013). The stable specific methane production rate was 612.8 mL/L.d at 25 ℃, which was

87.7% of the methane production rate at 35 ℃ (Table 4.4). The methane yield was 389.1 mL/g COD_r, which was around 90.5% of the 35 ℃ at 10 days of HRT. It is probable that the EAB utilized a little more energy for the cell maintenance at lower temperature condition, which is similar to anaerobic bacteria in a conventional anaerobic digester (Song et al., 2004; Varel et al., 1980; Zhang et al., 2006). In the case of the conventional anaerobic digestion, the methane production rate is considerably decreased at a low temperature condition. At 25 ℃, the methane production rate in conventional anaerobic digestion decreased to around 47.3-74.0% of that at 35 ℃, depending on the substrate type, pH adjustment and HRT (Zhang et al., 2006; Ghaly, 1996). This suggests that bioelectrochemical anaerobic digestion is less sensitive to temperature compared to conventional anaerobic digestion, which is attributable to the catalytic action of EAB related to the electrode potential. It is recognized that the activation energy required for an electrochemical reaction depends on electrode potential, as well as the catalyst (Protsenko et al., 2011; Rose et al., 2015; Anderson, 2003). On the other hand, the reaction kinetics in conventional anaerobic digestion are considerably influenced by temperature conditions. This is because the molecular fraction of the transient state reactants possessing enough kinetic energy to react is affected by temperature, according to the Maxwell-Boltzmann distribution law (Laidler, 1984). The methane content in biogas slightly decreased to 73.3% at 25 ℃. This indicates that the methane content was not sensitive to the temperature drop, and it was quite stable during the whole operation period at 25 ℃. From the above findings, the following hypotheses could be inferred to describe the recovery process of the bioelectrochemical digester exposed to temperature change: i) EAB activity is less sensitive to temperature change compared to the PMB, due to the contribution of electrode potential on the activation energy; ii) the methanogenic activity of EAB leads the recovering process in the early stage of the recovery; iii) the methanogenic activity of PMB is gradually recovered with time and then it is in excess of the EAB activity; and iv) the bioelectrochemical process is eventually stabilized.

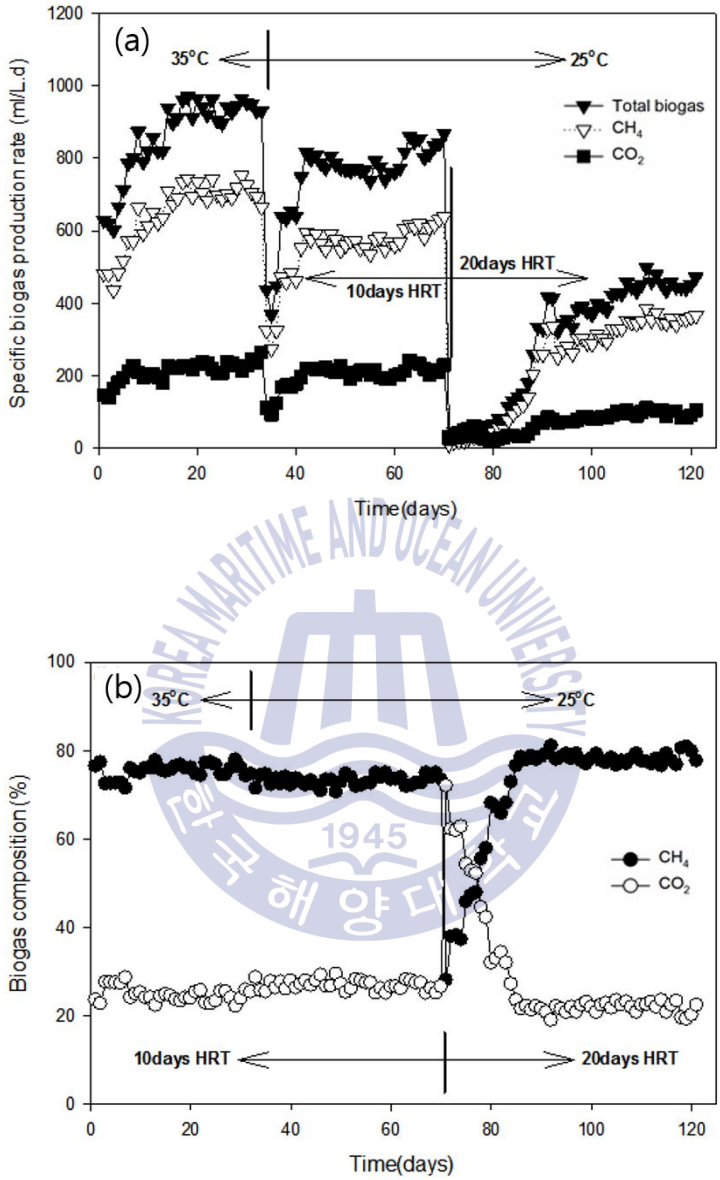


Fig. 4.7. (a) Bioelectrochmical biogas productions and (b) biogas compositions at different temperatures and HRTs.

Table 4.4 Performance of bioelectrochemical anaerobic digester at steady state in different temperatures and HRTs

Temperature (℃)		35	25	25
HRT (days)		10	10	20
Organic loading rate (g COD/L.d)		4.77±0.68	5.22±0.02	2.04±0.05
pH		7.35±0.01	7.02±0.01	7.14±0.00
Alkalinity (mg/L as $CaCO_3$)		5,162±46.6	3,553±28.2	4,040±4.1
VFAs	Level (mg COD/L)	386±41	680±30	620±23
	C2 (%):C3 (%)	85.1:14.9	64.7:35.3	50.7:49.3
VFAs/Alkalinity		0.075	0.191	0.153
SCOD		370±36.3	868±13.3	634±257.2
COD removal (%)		40.3±2.8	34.5±3.3	54.6±1.4
VS removal (%)		55.4±3.3	54.5±0.7	65.0±3.0
SMPR (mL/L.d)		698.6±5.6	612.8±6.0	349.7±3.4
CH_4 (%)		76.09±1.1	73.34±0.6	77.6±0.1
CH_4 yield (mL/g COD_r)		431.6±70.3	389.1±60.3	321.6±13.2
Potentials (V vs. Ag/AgCl) (anode/cathode)		-0.241/-0.541	-0.270/-0.570	-0.211/-0.521

SMPR: specific methane production rate

In the 71st day, the bioelectrochemical digester was disturbed by unintentional voltage shock to the electrodes for a few days. Methane production was abruptly dropped and not recovered for several days (Fig. 4.7a). It was obvious that the EAB on the electrode was seriously damaged by the voltage shock. High values in voltage and electric current are the emerging electrochemical approaches, which are used to control biofilm on conductive material surfaces (Sultana et al., 2015). After rectifying the voltage shock properly, the bioelectrochemical digester began operation again at 20 days of HRT without adjustment of the temperature condition. Then, methane production rate was slowly increased, though it took over 30 days to attain a stable methane production rate (Fig. 4.7a). This demonstrates that it takes a long time for the recovery of a bioelectrochemical anaerobic digester using damaged EAB as the inoculums. The specific methane production rate was stabilized at 349.7 mL/L.d (Table 4.4), which is 85.9% of the bioelectrochemical methane production rate at 35 ℃ and 20 days of HRT in

a previous study (Song et al., 2016). After the voltage shock, the methane content in the biogas was also gradually increased to 77.6% (Fig. 4.7b), which was similar to 77% of the methane content in biogas at 35 ℃ (Song et al., 2016). This suggests that temperature influence on methane production is similar at different HRTs ranging from 10 days to 20 days. At 20 days of HRT, the methane yield was 321.6 mL/g COD_r for 25 ℃ (Table 4.4), which was 82.7% of the yield at 10 days of HRT. It seems that the methane yield in the well-established anaerobic digester decreased at a dropped temperature and an extended retention time (Song et al., 2016; Varel et al., 1980; De la Rubia et al., 2002; Sunada et al., 2012).

② Organic matter removal (COD, VS)

Organic matter removal is one of the main concerns in the anaerobic digestion for sewage sludge. In a bioelectrochemical digester, the removal efficiencies in COD and VS were changed by the characteristics of feed sludge. However, the levels in COD and VS were also affected by disturbances of temperature and HRT (Fig. 4.8). After a disturbance, both COD and VS gradually stabilized like the methane productions. For 10 days of HRT, the stable value in COD removal efficiency was about 40.3% at 35 ℃, which was higher than 34.5% at 25 ℃ (Fig. 4.8a). This result is in agreement with higher methane production at 35 ℃, compared to 25 ℃. However, COD removal at 25 ℃ was increased to 54.6% by extending the HRT to 20 days (Fig. 4.8b). In the case of VS, the removal efficiency was 55.4% at 35 ℃, which was similar to 54.5% at 25 ℃. It is possible that a large fraction of the organic matter, which was produced from the hydrolysis of particulate organic matter and the subsequent acidogenesis, are the volatile forms in the bioelectrochemical digester at 25 ℃.

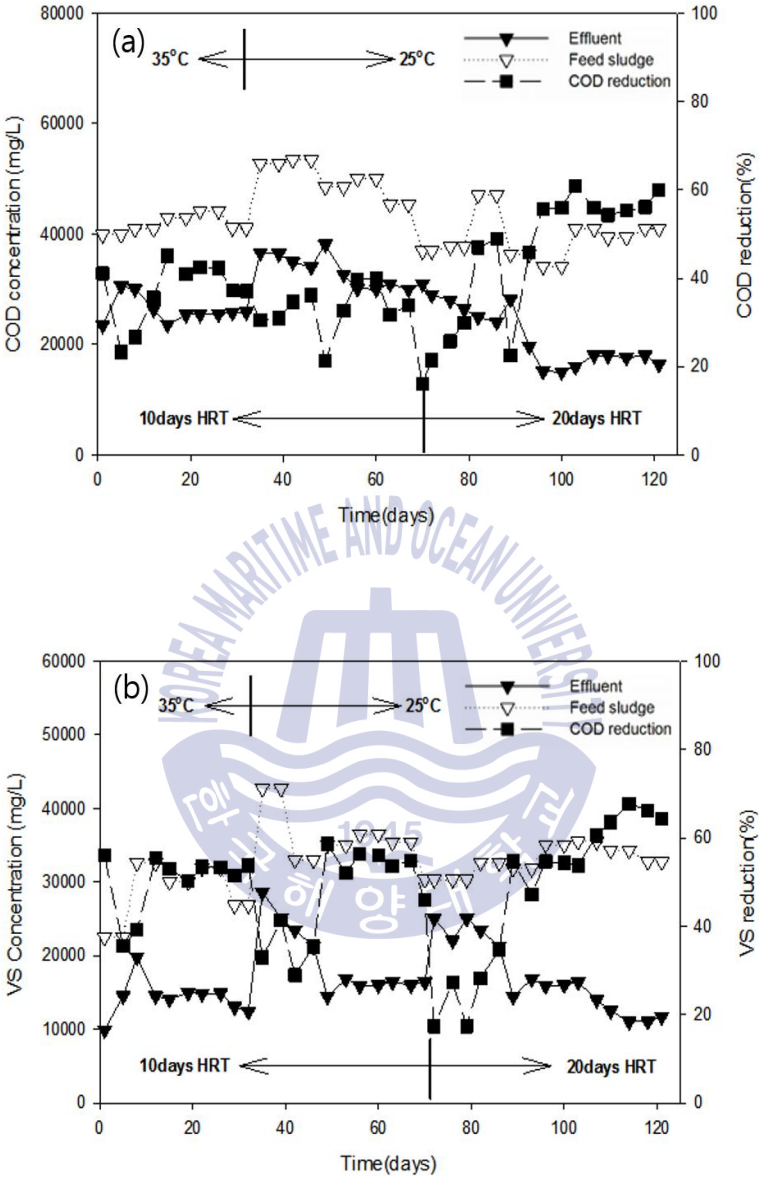


Fig. 4.8. Removal of (a) COD and (b) VS at different temperatures and HRTs.

This kind of uncoupling in the removal efficiency between COD and VS is commonly due to the accumulation of the volatile fatty acids by the imbalance between the consecutive conversion steps from the particulate organic matter into methane, and it is frequently reported in anaerobic digesters under different states (Song et al., 2016; Takashima et al., 2014; Astals et al., 2013; Silvestre et al., 2015). The VS removal was considerably increased to 65.0% at HRT of 20 days (25 ℃), similar with the increasing trend in COD removal. It seems that some particulate organic matter is additionally hydrolysed and fermented into VFAs at a longer retention time of 20 days. In a previous study, the additional hydrolysis of particulate matter in the bioelectrochemical digester was possible at longer retention times when intermediate concentrations such as monomers and VFAs, which are produced by hydrolysis and acidogenesis, were low (Song et al., 2016).

③ Energy efficiencies

Methane productions varying with conditions of temperature and HRT indicate differences in the energy outputs of bioelectrochemical anaerobic digestion. At 10 days of HRT, the methane energy output was 331.8 kJ at 35 ℃, which was higher than 291.0 kJ at 25 ℃ (Table 4.5). The methane energy output at 25 ℃ was only 166.2 kJ at 20 days of HRT. The energy inputs for bioelectrochemical digestion include the energy contained in the removed substrate, the electric energy input and the heating energy for feed sludge and maintenance of digester temperature. There are only small differences in the electric energy inputs ranged from 60 to 72 kJ while the energy contained in the removed substrate was considerably dependent on HRT and temperature conditions. In bioelectrochemical anaerobic digestion, the apparent energy inputs influencing methane production are electrical energy input, as well as the energy contained in the removed substrate. The maximum value of apparent energy efficiencies (EE) for the methane recoveries relative to the energy inputs were 96.1%, which was obtained at 35 ℃ and 10 days of HRT. However, the apparent energy efficiency at 10 days of HRT decreased

to 91.0% at 25 ℃, and decreased more to 65.3% at 20 days of HRT. Meanwhile, the heating energy for feed sludge to meet the mesophilic condition (35 ℃) was 100.6 kJ, higher than the ambient condition (25 ℃) (Table 4.5). Furthermore, the heating energy for feed sludge decreased in proportion to the decrease in HRT. This indicated that feed sludge heating is an important consideration in the estimation of energy efficiency for bioelectrochemical anaerobic digestion. At 10 days of HRT, the overall energy efficiency ($EE_{heating}$) considering feed sludge heating was 74.4% at 35 ℃, which is lower than 78.7% at 25 ℃, indicating that the operation of the bioelectrochemical digester at ambient temperature has some benefit in terms of overall energy efficiency, as well as VS removal. Furthermore, there are still some possibilities for more benefit in energy if heat losses through the walls, floor and roof of the digester were considered in temperate and cold climate regions, but further study is required. At 20 days of HRT, the overall energy efficiency was reduced to 59.4% at 25 ℃ due to the decreased methane yield.

Table 4.5 Energy efficiency of bioelectrochemical anaerobic digestion at different temperature conditions

Temperature (℃)	HRT (days)	W_{CH4} (kJ)	W_E (kJ)	W_s (kJ)	EE (%)	$W_{heating}$ (kJ)	Overall $EE_{heating}$ (%)
35	10	331.8	71.9	273.5	96.1	100.6	74.4
25	10	291.0	62.0	257.6	91.0	50.3	78.7
25	20	166.2	60.7	193.9	65.3	25.1	59.4

④ Behaviors of state variables (pH, Alkalinity, VFAs, SCOD and electrode potentials)

The observations for the responses in pH, alkalinity and VFAs to external disturbances give insight into the state of the bioelectrochemical anaerobic digester. The common behaviors in the anaerobic digester to disturbances are rapid pH drop by VFA accumulation and then gradual increase in pH, which is coupled with the recovery process (Song et al., Yuan et al., 2016; Vasquez et al., 2016). The recommended values in pH and alkalinity of anaerobic digestion for sewage sludge are 6.8-7.4 and 2,000-5,000mg/L as $CaCO_3$, respectively (Mao et al., 2015; Tchobanoglous et al., 2014). After start-up, the values of pH and alkalinity were gradually changed to around 7.35 and 5,200 mg/L as $CaCO_3$, respectively, without any serious variation (Fig. 4.9), indicating that the bioelectrochemical digester was well established at 35 ℃ and 10 days of HRT. When the temperature was downshifted to 25 ℃ on the 33rd day, the pH in the bioelectrochemical digester immediately dropped to 6.34 due to VFA accumulation, indicating that the methanogenic activity is more sensitive to the temperature drop compared to acidogenesis. However, pH gradually increased to 7.02 within 20 days without any alkalinity addition (Fig. 4.9a). The stable values in pH and alkalinity were slightly less than those at 35 ℃, which were in agreement with the decreased performance data in terms of methane production and COD removal efficiency. Thus, it was interesting that the pH recovery after the temperature downshift followed the methane production (Fig. 4.7a). In the case of the conventional anaerobic digestion, the methanogenic activity is seriously deteriorated at pH lower than 6.7 (Zhang et al., 2009a). In general, methanogenic activity is slowly recovered by maintaining the VFAs at low levels after adjusting the pH to neutral or higher values. It seems that the methanogenic activity of EAB rather than the PMB led the recovery process of the bioelectrochemical digester. In a previous study, the methanogenic activity of EAB was still high at pH 6.3 (Kim et al., 2015b). This demonstrates that the methanogenic activity of EAB in the bioelectrochemical digester is relatively less sensitive

to changes in environmental conditions such as pH and temperature. The change in alkalinity as a response to the temperature drop was similar to the pH in the outline. However, the behaviour in alkalinity was slightly different from pH in detail because the alkalinity is determined by several parameters such as feed sludge properties and intermediates such as carbon dioxide, ammonium and VFAs. In anaerobic digestion for sewage sludge, ammonium bicarbonate is an important buffering substance. Ammonium bicarbonate is produced from the anaerobic breakdown of nitrogenous organic compounds (Tchobanoglous et al., 2014), indicating higher alkalinity in the anaerobic digester and higher degradation of nitrogenous compounds. However, the alkalinity is mainly consumed by carbon dioxide as well as VFAs, which are produced from acidogenesis reaction.

In the 71st day, the bioelectrochemical digester was disturbed by unintentional voltage shock to the electrodes and subsequently changing the HRT to 20 days from 10 days. Methane production was considerably decreased for a long time (Fig. 4.7), indicating that the microbial activity in the bioelectrochemical digester was severely damaged. As an emergency measure, the pH was adjusted to over 7.0 by addition of sodium bicarbonate. Afterward, the values of pH and alkalinity were rapidly changed to 7.14 and 4,040 mg/L as $CaCO_3$, respectively, which were slightly higher than those at 10 days (Fig. 4.9). This indicates that the performance in the bioelectrochemical digester was slightly improved by the increased HRT of 20 days. It is considered that the rapid recovery in the pH and alkalinity were possible due to the slow recovery process of EAB activity at the low organic loading rate of 20 days HRT.

The total VFAs were closely linked with the levels in pH and alkalinity at different temperatures and HRTs (Fig. 4.10). When HRT was 10 days at 35 ℃, the total VFAs was the lowest as 386mg COD/L at 35 ℃. However, the VFAs increased to 680mg COD/L at 25 ℃, and slightly decreased to 620mg COD/L after HRT changed to 20 days. The ratio of total VFA to alkalinity is also an indicator of anaerobic digester stability. The ratio of total VFA to

alkalinity for a stable anaerobic digester is in the range of 0.1 to 0.3, and a ratio over 0.4 indicates an instable state of the anaerobic digester usually involving VFA accumulation (Song et al., 2016; Padilla-Gasca et al., 2011). At 10 days of HRT, the ratio of total VFA to alkalinity in the bioelectrochemical digester was 0.075 at 35 ℃, and it increased to 0.191 at 25 ℃. However, the ratio was reduced to 0.153 again at 25 ℃ when the HRT was increased from 10 days to 20 days. These results indicate that the bioelectrochemical digester is very stable at 25 ℃ as well as at 35 ℃. The VFA composition in the anaerobic digester gives more information on the state of the anaerobic digester. During operation of the bioelectrochemical anaerobic digester, the main components of short chain VFAs were acetic acid and propionic acid, but their percentages were affected by the operation temperatures and the HRTs (Table 4.4). At 10 days of HRT, the main component was HAc (85.1%) at 35 ℃, but it reduced to 64.7% at 25 ℃. In a previous study, the optimum temperature for the propionic acid-producing bacteria such as Paludibacter Propionicigenes gen. nov., sp. nov. was around 30 ℃ (Song et al., 2016; Ueki et al., 2006). It is probable that the growth rate of propionic acid-producing bacteria is higher at 25 ℃ than the 35 ℃. The propionic acid portion was increased to 49.3% when the HRT was increased to 20 days. At 20 days of HRT, the higher percentage of propionic acid in the VFA indicates the increased retention of the propionic acid-producing bacteria. In a conventional anaerobic digester, the accumulation of propionic acid is generally observed in a perturbation period such as the start-up Vasquez et al., 2016; Regueiro et al., 2014), but the methane production did not seem significantly reduced at 25 ℃ compared to conventional anaerobic digestion (Mao et al., 2015; Connaughton et al., 2006). The main components of SCOD in an anaerobic digester are the monomers from the hydrolysis and the VFAs acidified from the monomers, which is consumed by the conversion of the VFAs into methane. In the present study, similar trends with VFAs were observed in the SCOD values. At 10 days of HRT, the SCOD was 370mg/L at 35 ℃, and it was slightly increased to 868mg/L at 25 ℃. However, the ratio of VFAs to SCOD were

in the range of 0.72-0.78. These results indicate that the bioelectrochemical digester was stable by balancing between the hydrolysis and acidogenesis and the methanogenesis. At 20 days of HRT, the SCOD was slightly reduced to 634 mg/L at 25 ℃, but the ratio was increased to 0.98, indicating that hydrolysis was the rate limiting step in the whole bioelectrochemical anaerobic digestion at the longer HRT of 20 days.

In a bioelectrochemical anaerobic digester, the electrode potentials are an important factor influencing the bioelectrochemical reactions for methane production from organic matter degradation (Kim et al., 2015b). During the operation of the bioelectrochemical digester, the potential difference between anode and cathode was set to 0.3V using an external DC power source. However, the electrode potentials versus the reference electrode (Ag/AgCl) were different depending on the conditions of temperature and HRT. Then, the thermodynamic limit value of cathode potential is -0.445V (vs. Ag/AgCl) for carbon dioxide conversion into methane (Song et al., 2016; Kim et al., 2015b). At 10 days of HRT, the cathode potential was -0.541V (vs. Ag/AgCl) at 35 ℃, but it was shifted to the negative value (-0.570V vs. Ag/AgCl) at 25 ℃ (Fig. 4.11). The electrode potentials in a bioelectrochemical digester are determined by the difference between the oxidation rate on the anode and the reduction rate on the cathode (Song et al., 2016). The shift of electrode potentials in a negative direction indicates that the oxidation rate is higher than the reduction rate. This means that the reduction rate of carbon dioxide to methane limits the whole bioelectrochemical reactions at 25 ℃ unlike that at 35 ℃. However, at 20 days of HRT, the cathode potential was moved to more positive value (-0.511V vs. Ag/AgCl), and the ratio of VFAs to SCOD was also quite high at 0.98, indicating that bioelectrochemical methane production was limited by the hydrolysis step. This suggests that the bioelectrochemical reaction at the ambient temperature of 25 ℃ is controlled by the hydrolysis of particulate organic matter at longer HRT of 20 days with lower organic loading rate.

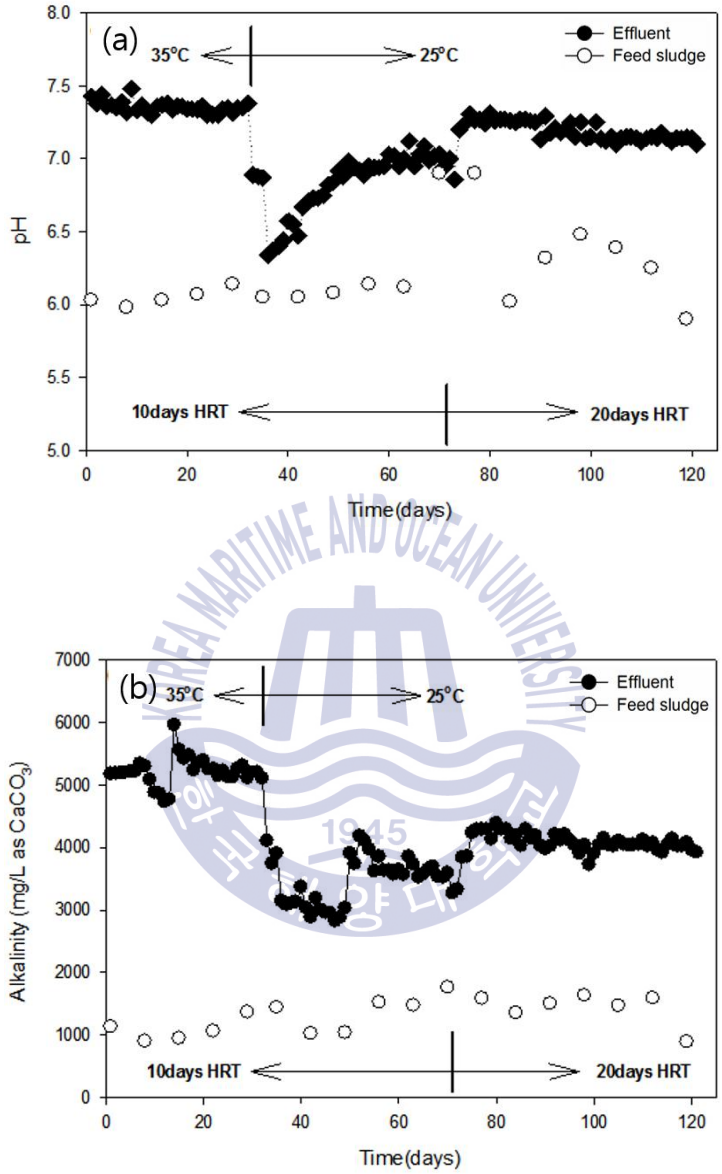


Fig. 4.9. Behaviors of (a) pH and (b) alkalinity at different temperatures and HRTs.

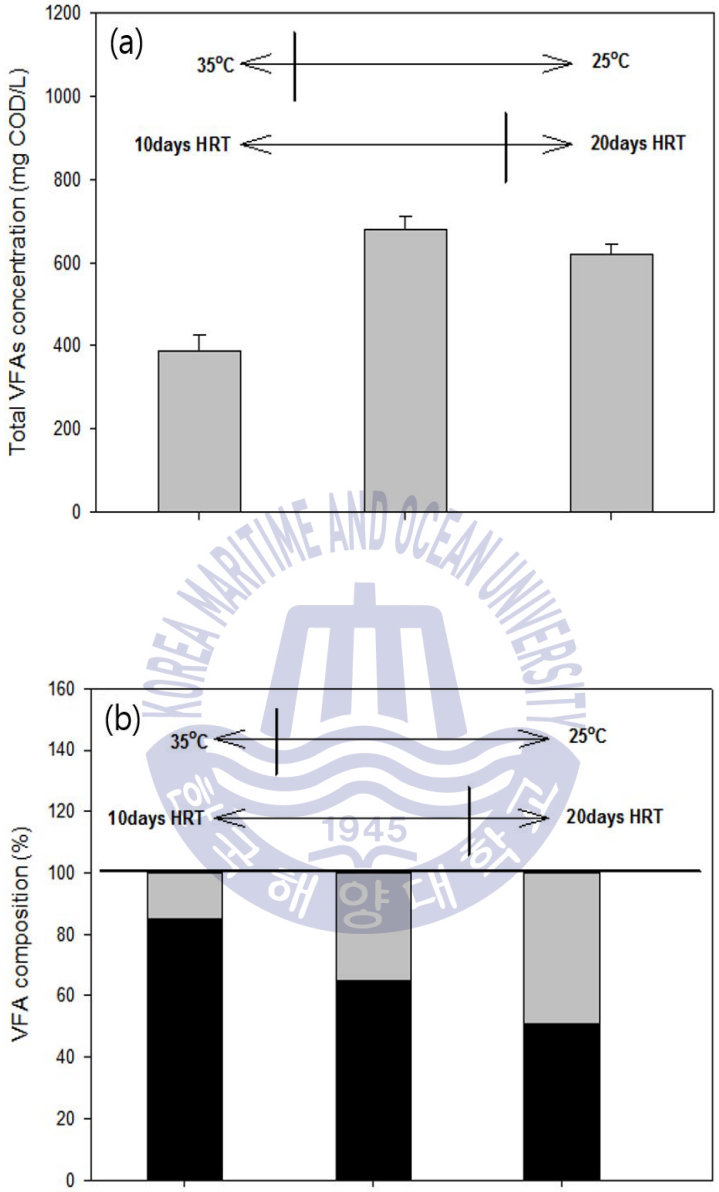


Fig. 4.10. (a) Total VFAs and (b) VFA compositions at different temperatures and HRTs.

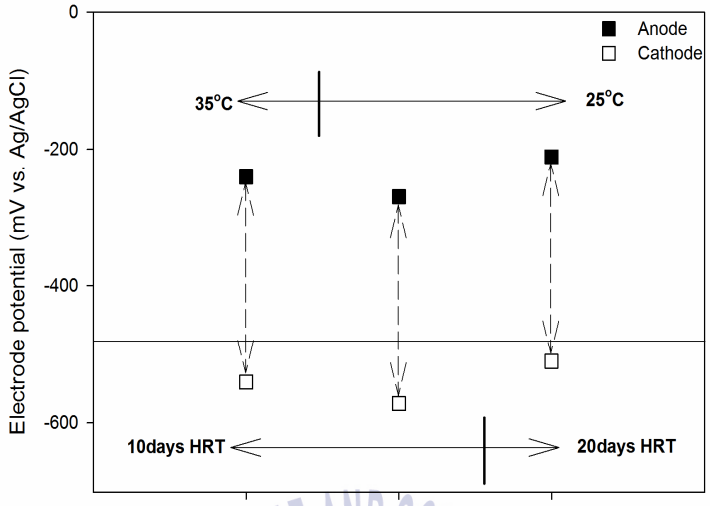


Fig. 4.11. Potentials of anode and cathode at different temperatures and HRTs.

⑤ Implications of ambient temperature operation

Anaerobic digestion for sewage sludge is a common approach for recovery of methane as a by-product, as well as organic matter stabilization. The large portion of the organic matter contained in the sewage sludge is converted into methane during anaerobic digestion. However, the heating energy for the sewage sludge, which is required for maintaining the digestion temperature, is essential for anaerobic digestion. This means that the performance of an anaerobic digester is expressed as energy efficiency, as well as the removal efficiency of organic matter in terms of COD or VS. In conventional anaerobic digestion for sewage sludge, the digester is commonly operated under mesophilic condition (35 ℃) at over 20 days of HRT, but the removal efficiency in VS is not satisfactory at around 30-45% (Song et al., 2004; Takashima et al., 2014; Tchobanoglous et al., 2014; Peces et al., 2013). The methane yield based on the COD removed is about 123-352 mL g COD_r, indicating that energy efficiency, based on the theoretical methane yield, can be reduced to around 35% (Takashima et al., 2014; Astals et al., 2013).

However, energy efficiency would be reduced more if the large heating energy required for the sewage sludge is considered in the case of cold climate regions. The temperature of raw sewage sludge depends on the air temperature. In the bioelectrochemical digester at 35 ℃ for sewage sludge, the removal efficiency in VS was as high as 70.5% at 20 days of HRT, and the apparent energy efficiency, based on the methane recovery relative to both the substrate removed and the electrical energy input, was 69.1% (Song et al., 2016). In the present study, the VS removal in mesophilic condition (35 ℃) was 55.4% at 10 days of HRT (Table 4.4), but the energy efficiency was improved to 98.8%. This indicates that energy efficiency is improved at shorter HRT or higher organic loading rates, but the removal efficiency in organic matter is reduced. Interestingly, when the temperature was reduced to 25 ℃, the overall energy efficiency considering the heating energy for the feed sludge, as well as the removal efficiency in VS were similar to those at 35 ℃. This indicates that overall energy efficiency would be improved more if the heat losses through the digester wall, roof and bottoms are considered in cold climate regions. Meanwhile, the performance in VS removal at 25 ℃ was considerably improved by extending the HRT to 20 days. As aforementioned, the conventional anaerobic digester could be easily converted to a bioelectrochemical digester by installing electrodes inside of the digester and then maintaining a small difference in potentials, ca. 0.3V, between the electrodes. The findings in this study are valuable for the anaerobic digestion of organic waste in moderate and cold climate regions where large heating energy is required to maintain mesophilic condition for an anaerobic digester.

4.3.3 Influence of applied voltage on bioelectrochemical anaerobic digestion

① Bioelectrochemical methane production

In a bioelectrochemical anaerobic digester, the applied voltage between the anode and cathode is a major parameter that determines the electrical potentials of the electrodes. The methanogenic activity of EAB in the bioelectrochemical digester depends on the electrical potentials of the electrodes. At ambient temperature (25±2 ℃), the biogas production rate and methane content in our experiment were considerably affected by the applied voltage (Fig. 4.12). At 0.5V of applied voltage, the methane production rate gradually increased after the initial acclimation time, and the cathode potential was also slowly stabilized at -0.73V (vs Ag/AgCl) (Table 4.6). The biogas production rate was stable at 346±15 mL CH_4/L.d, and the methane content in the biogas was as high as 80.6% (Table 4.7). In mesophilic conventional anaerobic digestion for sewage sludge, the methane production rate and methane content are is approximately 250 mL CH_4/L.d and 58.7-62.8%, respectively (Gavala et al., 2003; Mottet et al., 2010). This indicates that the bioelectrochemical anaerobic digestion at 0.5V of applied voltage has better performance at ambient temperature, compared to the conventional anaerobic digestion.

In a bioelectrochemical anaerobic digester, the biogas sources are the activities of EAB and the PAB (Koch et al., 2015; Song et al., 2016). The EAB growing on the cathode surface produces methane as a main product from the reduction of carbon dioxide. The other species of EAB such as *Shewanella Oneidensis MR-1* and *Geobacter sulfurreducens* on the anode surface secrete some endogenous electron shuttles such as riboflavin and riboflavin-5'phosphate (Song et al., 2016; Wu et al., 2013; Yang et al., 2012), which enhance the methanogenic activity of PAB (Song et al., 2016). The methane content in the biogas produced from the methanogenic activity of PAB is commonly determined by the types of substrates, as well as some

operational and environmental parameters such as pH, temperature, and HRT. In conventional mesophilic anaerobic digestion for sewage sludge, the methane content in biogas is approximately 50-65% and is produced by the methanogenic activity of PAB (Shin & Song, 1995; Song et al., 2004). This suggests that the biogas production and methane content in the bioelectrochemical digester are determined by the relative contributions of the EAB and PAB. The above results indicate that the methanogenic activity of EAB at ambient temperature is good at -0.73V (vs. Ag/AgCl) of the cathode potential. In the previous study, the proper cathode potential at mesophilic condition ranged from -0.546V (vs. Ag/AgCl) to -0.61V (vs. Ag/AgCl) (Kim et al., 2015b). This demonstrates that the proper electrical potential for the methanogenic activity of EAB on the cathode is shifted to a slightly more negative value at ambient temperature, compared to the mesophilic condition. After adjusting the applied voltage to 0.7V, the cathode potential was changed to a more negative value of -0.96V (vs. Ag/AgCl). The methane content in the biogas quickly dropped to 22%, and then slowly recovered up to 57.6% (Fig. 4.12b). The specific methane production rate also decreased to around 56 mL CH_4/L.d. It is possible that the methanogenic activity of EAB was lost due to the more negative value (-0.96V (vs. Ag/AgCl)) of the cathode potential, and the methane was mainly produced from the methanogenic activity of PAB rather than the EAB. The theoretical potential of the cathode for hydrogen production is -0.61V (vs. Ag/AgCl) (Hamelers et al., 2010; Wang et al., 2009). It seems that -0.96V (vs. Ag/AgCl) of the cathode potential is a favorable condition for hydrogen production. After decreasing the applied voltage to 0.3V, the electrical potential gradually stabilized at -0.51V (vs. Ag/AgCl). Then, the specific methane production rate increased to 370±7 mL CH_4/L.d, which was higher than that at 0.5V of applied voltage. However, the methane content in the biogas was slightly lower at 77.7%, compared to 80.6% at 0.5V of applied voltage. This indicates that the methanogenic activity of PAB improved more than the activity of EAB at 0.3V of applied voltage.

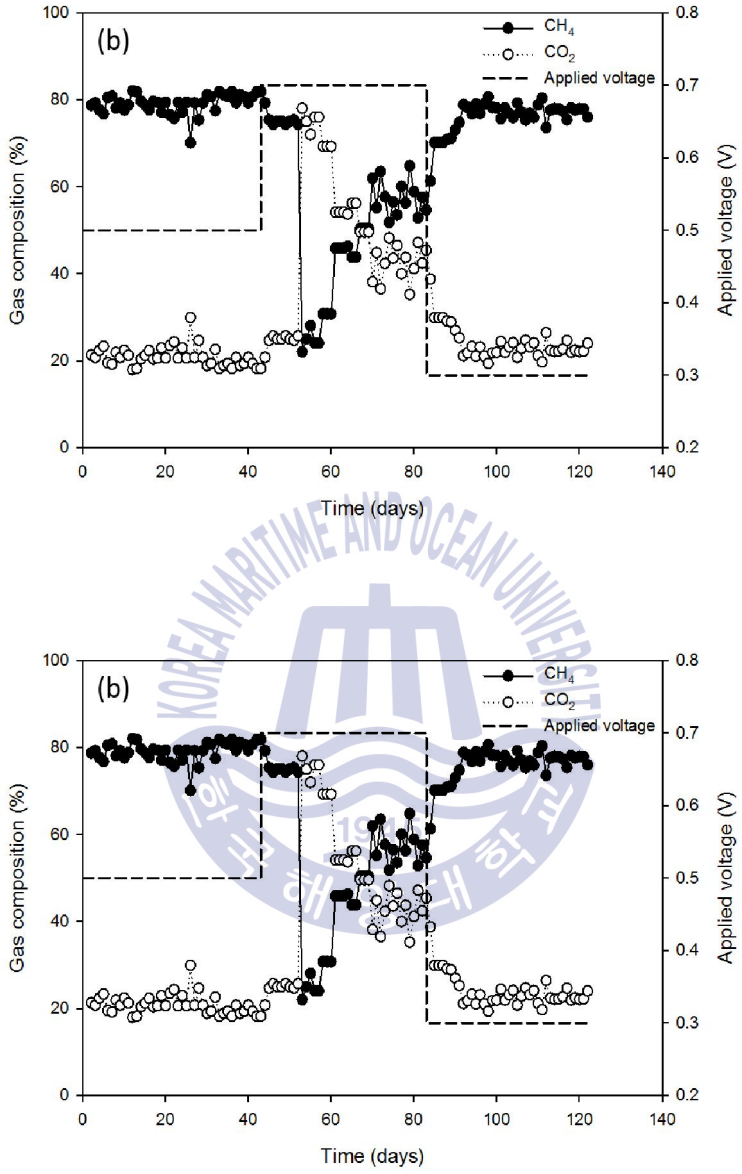


Fig. 4.12. Changes of (a) bioelectrochemical methane production and (b) biogas compositions in biogas at the applied voltage from 0.3V to 0.7V.

Table 4.6 Properties of bioelectrochemical anaerobic digester for sewage sludge at the applied voltages from 0.3V to 0.7

State variables \Voltage (V)		0.3V	0.5V	0.7V	CM-AD
pH		7.13±0.05	7.15±0.06	6.68±0.12	7.2-8.0 (Kardos et al., 2011)
Alkalinity (mg/L as $CaCO_3$)		3,992±131	4,233±205	3,012±162	4,000-6,000 (Kardos et al., 2011)
SCVFAs (mg COD/L)		405±23	463±50	1,584±200	618 (Song et al., 2004)
TVFAs/Alkalinity		0.15±0.01	0.14±0.04	0.63±0.07	0.27-0.67 (Kardos et al., 2011)
SCOD (mg/L)		620±136	630±102	2,213±649	1,242-7,939 (Chen et al., 2007)
Potentials (V vs.Ag/AgCl)	Anode	-0.21±0.03	-0.23±0.05	-0.25±0.02	-
	Cathode	-0.51±0.02	-0.73±0.04	-0.96±0.02	-
Current density (mA/m^3)		195±1.5	294±2.3	392±3.1	-

CM-AD: conventional mesophilic anaerobic digester

② Organic matter (COD, VS) removal and energy efficiency

The total COD in substrate sewage sludge varied in the range of 31,706-47,017 mg/L, and the effluent COD was affected by the applied voltage as well as the COD in the sewage sludge (Fig. 4.13a). The COD removal efficiencies at 0.3V and 0.5V of applied voltage were similar at about 54-56% at the steady state. However, the total COD removal efficiency was significantly reduced to 32.6% at 0.7V of applied voltage, which is similar with the range of 31-46% for conventional mesophilic anaerobic digestion (Takashima & Tanaka, 2014) (Table 4.7). The COD removal efficiencies were in agreement with the methane productions at different applied voltages. The VS levels of the raw sewage sludge varied in the range of 43,300-51,000 mg/L (Fig. 4.13b). The removal behaviors of VS

were similar to the changes of the COD (Fig. 4.13a), indicating that the removals of VS were also affected by the applied voltage (Fig. 4.13b). The VS removal efficiency was considerably high at about 64-66% at 0.3V and 0.5V of the applied voltages. In the conventional mesophilic anaerobic digestion of sewage sludge, the VS removal efficiency was 32.1-46.1% (Kim et al., 2003; Song et al., 2004). However, at 0.7V of applied voltage, VS removal was reduced to 31.0%. This is because a large portion of the soluble organic matter, which was not converted into methane, is accumulated in the bioelectrochemical digester.

The methane yield estimated as the methane production (mL CH_4) per g COD removed was 350 mL CH_4/g COD_r at 0.5V of applied voltage, followed by 330 mL CH_4/g COD_r at 0.3V (Table 4.7). In the bioelectrochemical anaerobic digester, the methane yield depends on the contributions of the EAB and PAB to the total methane production. In previous studies, the coulomb efficiency for the bioelectrochemical synthesis of products such as methane is as high as 70-96% (Liu et al., 2016; Xafenias & Mapelli, 2014). However, in the conventional mesophilic anaerobic digester, the methane yield was 123-263 mL CH_4/g COD_r, which was around 35-75% of the theoretical value (350mL CH_4/g COD_r for glucose) (Mottet et al., 2010). At 0.5V of applied voltage, higher methane yield is in agreement with higher methane content in biogas, compared to that at 0.3V of applied voltage. At 0.7V of applied voltage, the methane yield was only 162 mL CH_4/g COD_r, which probably imputed the loss of the methanogenic activity of EAB at -0.96V (vs. Ag/AgCl) of the cathode potential, as well as the low methanogenic activity of PAB. The energy efficiency was also estimated as the recovered energy as methane relative to the electric energy input and the energy content in removed organic matter as COD. Energy efficiency was dependent on the applied voltage, but it was slightly different from the methane yield. The energy efficiency was the highest at 63.0% at 0.3V of applied voltage, which was higher than 43.9% at 0.5V (Table 4.7). It is likely that energy efficiency is higher if the methane

production from the activity of PAB is higher than the portion from EAB. At 0.7V of applied voltage, the energy efficiency was only 7.0%. This indicates that EAB lost almost all methanogenic activity at -0.96V (vs. Ag/AgCl) of the cathode potential. This also suggests that the coulomb efficiency in a bioelectrochemical system is significantly decreased at higher applied voltage over the proper value of the electrode potential. At mesophilic condition, the energy efficiency for the bioelectrochemical anaerobic digestion of sewage sludge was 69.1% at 0.3V of applied voltage (Song et al., 2016). This indicates that the activity of EAB to produce methane requires more energy at ambient temperature than at mesophilic condition.

Table 4.7 Performance of bioelectrochemical anaerobic digester for sewage sludge at the applied voltages from 0.3V to 0.7V

Potential difference (V)	VS removal (%)	TCOD removal (%)	Specific CH_4 production (mL CH_4/L.d)	CH_4 content (%)	Methane yield (mL CH_4/g COD_r)	Overall energy efficiency (%)
0.3V	65.9±2.2	55.4±2.1	370±7	77.3±0.9	330±27	62.99
0.5V	64.1±2.3	54.5±2.8	346±15	80.6±1.1	350±28	43.91
0.7V	31.0±2.2	32.6±8.0	56±9	57.6±3.7	162±50	7.03
CM-AD	32.1-46.1 (Kim et al., 2003; Song et al., 2004)	31-46 (Takashima & Tanaka, 2014)	250 (Gavala et al., 2003)	58.7-62.8 (Mottet et al., 2010)	123-263 (Mottet et al., 2010)	

CM-AD: conventional mesophilic anaerobic digester

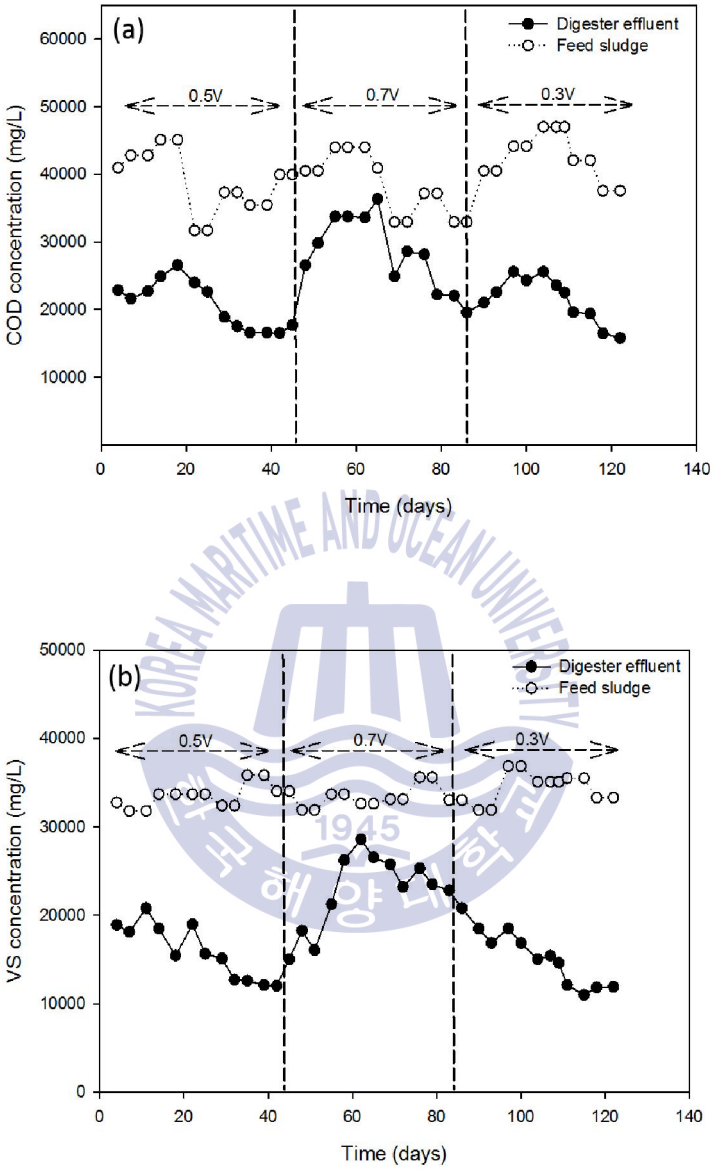


Fig. 4.13. Behaviors of (a) COD and (b) VS in bioelectrochemical anaerobic digester at the applied voltages from 0.3V to 0.7V.

③ Process state variables (pH, alkalinity, VFAs and SCOD)

In the bioelectrochemical digester, the buffering capacity against pH fluctuation is mainly supplemented by both activities of EAB for the anodic oxidation of VFAs and for the cathodic reduction of carbon dioxide into methane (Koch et al., 2015; Song et al., 2016). After the start-up at 0.5V of applied voltage, pH was maintained at around 7.15 (Fig. 4.14a). As the applied voltage was changed to 0.7V, the pH became unstable and dropped to 6.34. The pH then slowly recovered to 6.68 after several days of operation. The instability and decrease in pH at 0.7V of applied voltage were probably imputed to the loss of EAB activities. After adjusting the applied voltage to 0.3V, the pH gradually recovered to nearly 7.13 (Table 4.6). In the previous study at mesophilic condition (Song et al., 2016), the pH in the bioelectrochemical digester was 7.13-7.32, which was influenced by both the applied voltage and HRT.

Meanwhile, the alkalinity was stabilized to 4,233 mg/L as $CaCO_3$ at 0.5V of applied voltage. The normal value of alkalinity in conventional anaerobic digestion ranged from 4,000 to 6,500 mg/L as $CaCO_3$ (Kardos et al., 2011; Song et al., 2016) (Fig. 4.14b). When the applied voltage was changed to 0.7V, the alkalinity decreased to 3,012 mg/L as $CaCO_3$. The alkalinity generally increased from the acetoclastic methanogenesis and the sulfate reduction, as well as ammonium ion from the degradation of nitrogenous compounds, and it decreased due to VFA accumulation. It seems that the methanogenic activity for alkalinity production decreased, and VFA accumulated as -0.96 (vs. Ag/AgCl) of the cathode potential was too negative. However, at 0.3V of applied voltage, alkalinity quickly increased to 3,992 mg/L as $CaCO_3$, which was similar to the increase in methane content in biogas rather than the methane production rate or pH recovery. This demonstrates that the increase in alkalinity and methane content in biogas are closely linked with the activity of EAB.

The concentration of volatile fatty acids (VFAs) is an important indicator informing the balance of several biochemical reaction steps. At 0.3 and 0.5V

of applied voltage, VFA levels were as low as 405-460 mg COD/L, which were 65.3-73.5% of the SCODs, and lower than the conventional mesophilic anaerobic digestion(Song et al., 2004) (Table 4.6). This indicates that the anaerobic degradations of the substrate were well balanced and performed at 0.3V and 0.5V of applied voltage (Fig. 4.15a). However, VFA accumulated up to 1,584 mg COD/L at 0.7V of applied voltage, but the percentage of VFA contained in SCOD were similar to those at 0.3V and 0.5V of applied voltage. This indicates that the anaerobic degradation was limited by the methanogenic steps. The optimal ratio of total VFA to alkalinity for anaerobic digestion is in the range of 0.1 to 0.3, but it's easily increased to high value as 0.27-0.67 in conventional mesophilic anaerobic digester (Kardos et al., 2011; Song et al., 2004). In this study, TVFA/alkalinity ratio was 0.14-0.15 at 0.3V and 0.5V of applied voltage, but the ratio increased to 0.63 at 0.7V, which was caused by VFA accumulation and low methanogenesis activity (Table 4.6).

VFA composition was also affected by the applied voltage (Fig. 4.15b). The main components of VFAs were acetic and propionic acids at 0.3 and 0.5V of applied voltage, but at 0.7V, formic acid portion considerably increased to around 43.9% rather than decrease in the portions of acetic acid and propionic acid. The formic acid is produced from Co-A-dependent cleavage, carbon dioxide reduction in NADH, or ferrodoxin-dependent manner, and the formic acid is an important substrate for syntrophic methanogens through interspecies electron transfer (Dolfing et al., 2008). However, the conversion of formic acid into methane is inhibited by high hydrogen partial pressure. It seems that at 0.7V of applied voltage, the formic acid accumulated due to increased hydrogen partial pressure or decrease in the activity of syntrophic methanogens.

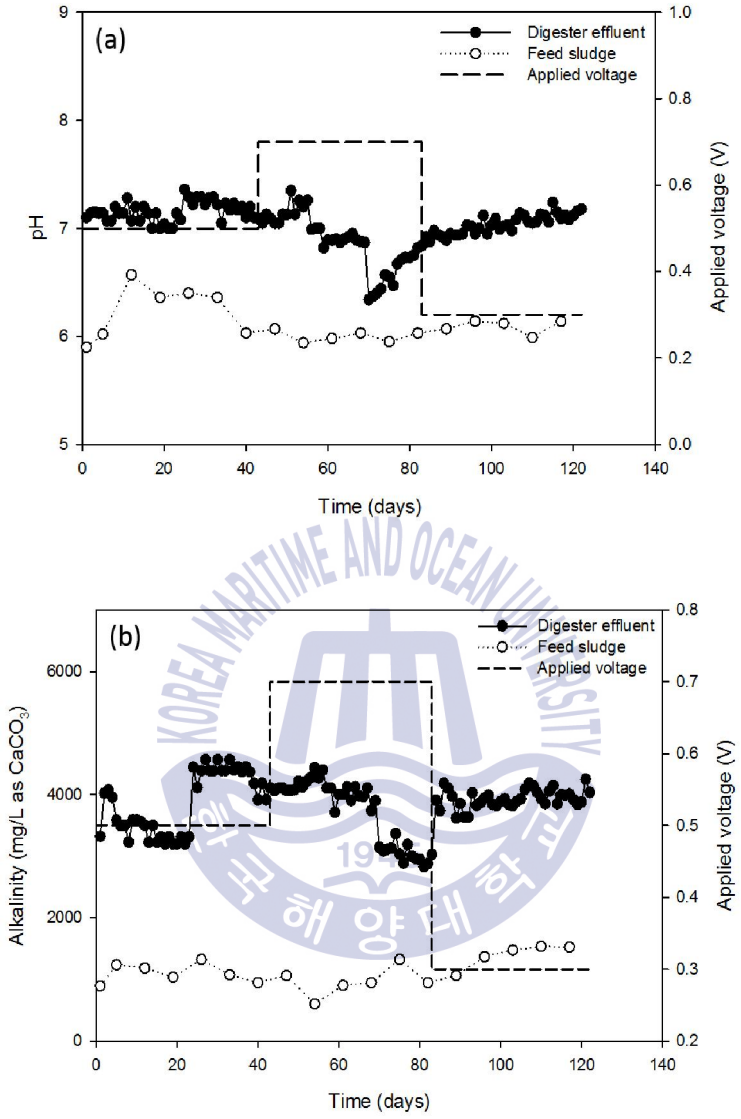


Fig. 4.14. Changes of (a) pH and (b) alkalinity in bioelectrochemical anaerobic digester at the applied voltage from 0.3V to 0.7V.

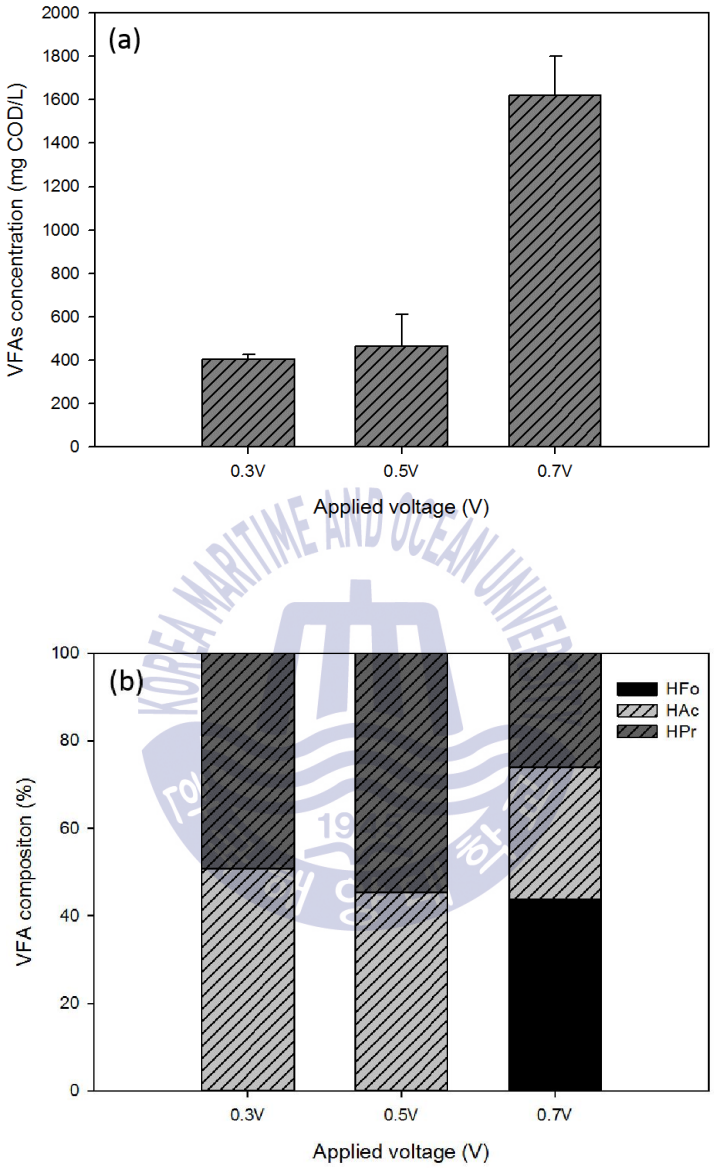


Fig. 4.15. Levels (a) and Compositions (b) of VFAs in bioelectrochemical anaerobic digester at the applied voltage.

④ Planktonic microbial communities

The applied voltage had an influence on the microbial communities of PAB (Fig. 4.16). At 0.3V and 0.5V of applied voltage, there are some similarities in the distribution of the microbial community for PAB, which was quite different at 0.7V of applied voltage. This indicates that the performance in the bioelectrochemical anaerobic digester was closely linked to the microbial communities for PAB. Fig. 4.17 illustrates the phylum, class and species distributions of bacteria involved in 0.3V, 0.5V, and 0.7V of applied voltage. The main phyla in the bioelectrochemical anaerobic digester were *Porteobacteria, Chloroflexi* and *Firmicutes*. *Proteobacteria* as a dominant phylum was 31.0% at 0.3V and 29.9% at 0.5V, but it was reduced to 23.0% at 0.7V of applied voltage (Fig. 4.17a). Especially, *Cloacamonas_p,* one of the main phyla, was around 10% at 0.3 and 0.5V, but it was reduced to 0.38% at 0.7V. Fig. 4.17b shows the distributions in class level at different applied voltages. The two dominant classes were *α-proteobacteria* and *β-proteobacteria*, and the portion of these two classes was 26.1% and 23.9% at 0.3V and 0.5V, respectively, and 18.1% at 0.7V of the applied voltage. Fig. 4.17c shows the distributions in the species in the microbial communities. At 0.3V and 0.5V of applied voltage, *Cloacamonas* was the most dominant species with a value of 7.63% and 8.03%, respectively, but at 0.7V, it was reduced to 0.13%. It is well known that *Cloacamonasis* a bacterium belonging to the *synergistetes* group, which is a dominant species in anaerobic digester sludge, and *Cloacamonas acidaminovorans* is related to the acetate and propionate degradations and the alkalinity production (Juste-Poinapen, 2015; Pelletier et al., 2008; Sieber et al., 2012). It seems that the reduction of the methane production at 0.7V of applied voltage is mainly imputed to the reduction of *Cloacamonas*. However, the dominant species at 0.7V was *Saprospiraceae* (3.46%), which were only 0.99% and 1.36% at 0.3V and 0.5V of applied voltage, respectively. The portion of *Saprospiraceae, Fimbriimonas* and *Ottowia pentelensis*, which are well known as hydrolytic bacteria, was 1-3 times higher at 0.7V than those at 0.3V and

0.5V of applied voltage. These results indicate that the reduced pH, alkalinity and methane production at 0.7V of applied voltage was ascribed to the reduction of methanogenic activity rather than the reduction in hydrolysis and acidification.

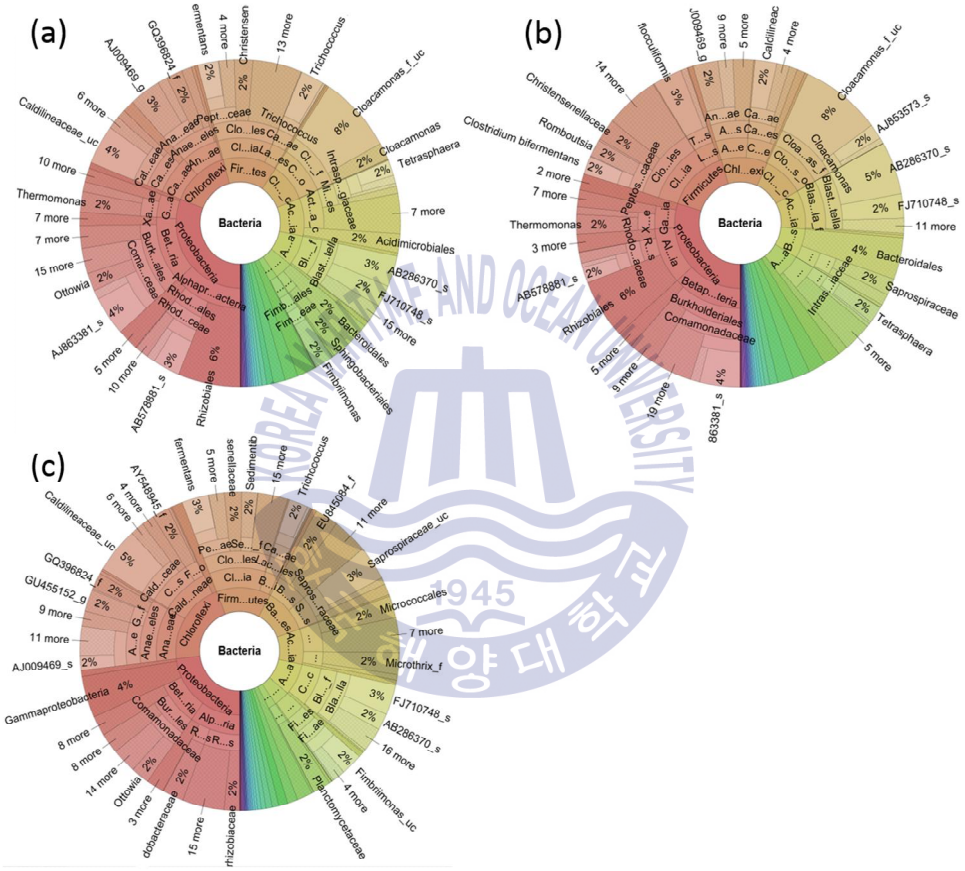


Fig. 4.16. Pie charts showing the percentage of abundance of the phylum with species level of the planktonic microbial communities (a) 0.3 V, (b) 0.5 V, (c) 0.7 V.

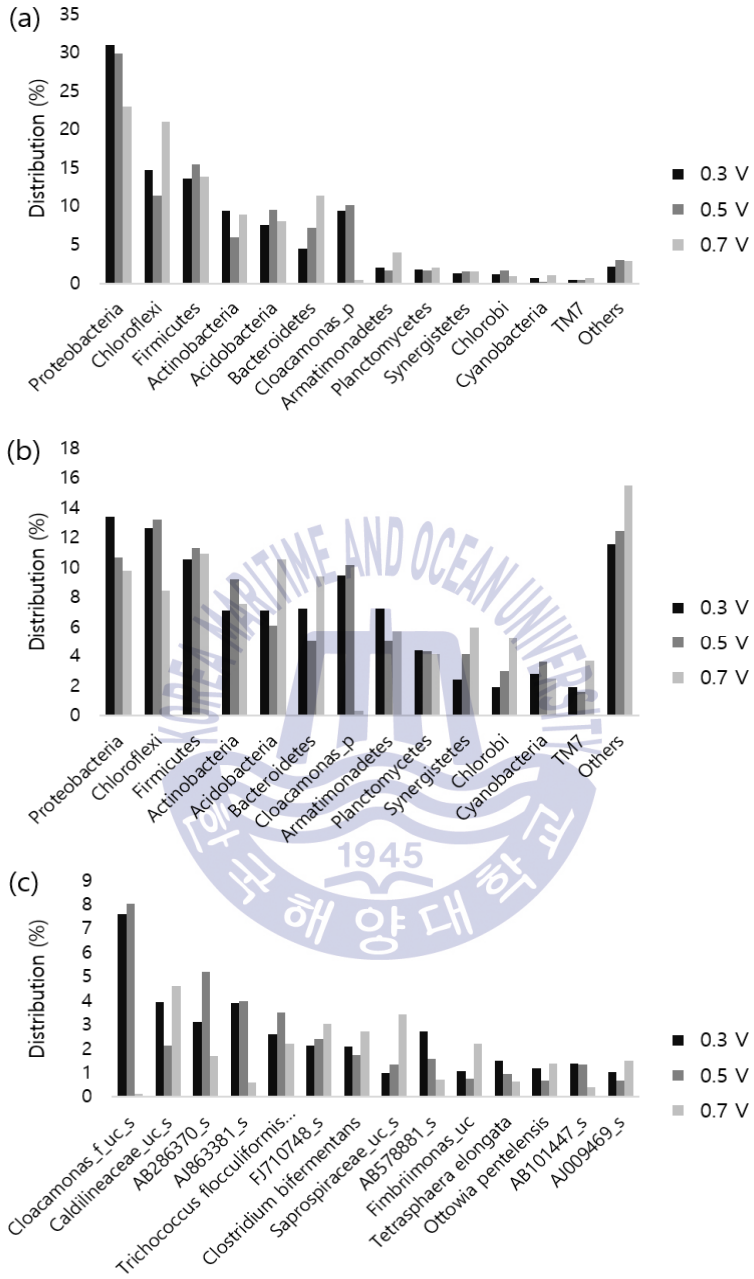


Fig. 4.17. Percentage of (a) phylum, (b) class and (c) species for planktonic microbial communities at the applied voltages from 0.3 V to 0.7 V.

4.4 Conclusions

Boosting low voltage of 0.3V could improve the bioelectrochemical anaerobic digestion of sewage sludge at relatively short HRT ranges of 5 to 20 days. At 20 days of HRT condition, VS removal (70.5%), specific methane production rate (407.0 mL CH_4/L.d), and biogas methane content (76.9%) were much higher than that could be obtained with conventional anaerobic digestion processes. The maximum methane production rate was 1,339 mL CH_4/L.d which was obtained shorter HRT of 5 days, while the methane content and VS reduction decreased slightly. The overall energy efficiency based on methane recovery was 69.1%-98.7%, and the maximum value was obtained at HRT of 10 days. These results demonstrate that boosting small voltage (0.3V) to anaerobic digestion system can increase energy efficiency of the system as well as VS reduction without any inhibitory effects.

A comparison of bioelectrochemical methane production from sewage sludge at ambient temperature to mesophilic condition was studied at different HRTs. At 10 days of HRT, the mesophilic bioelectrochemical digester (35 ℃) was very stable with a high VS removal efficiency of 55.4%, and the methane production and methane content in the biogas were 698.6 mL/L.d and 76.1%, respectively. For the temperature downshifted to ambient temperature (25 ℃), the bioelectrochemical digester quickly adapted within 10 days, and a stable operation of the digester was possible. At ambient temperature, the methane production and methane content in the biogas were slightly lower than those at 35 ℃. However, at ambient temperature, the VS removal efficiency are similar to those at 35 ℃, and the energy efficiency was more higher by considering the heating energy. By extending the HRT to 20 days, bioelectrochemical digestion at 25 ℃ become more stable and the performance in VS removal also improved compared to that at 10 days of HRT. The bioelectrochemical anaerobic digestion is recommended for the methane production from organic waste in moderate or cold climate regions.

In bioelectrochemical anaerobic digestion, the process performance and methanogenic activity (EAD EAB & PAB) at ambient temperature (25±2 ℃)

is affected by the applied voltage. The process stability and performance in terms of organic matter removal and methane production are better at both 0.3V and 0.5V of applied voltage, but it's poor at 0.7V of applied voltage due to the accumulation of VFAs and the decrease of pH. The dominant species of PAB in the suspended sludge was *Cloacamonas* at 0.3V and 0.5V, but hydrolytic bacteria such as *Saprospiraceae, Fimbriimonas*, and *Ottowia pentelensis* are dominant at 0.7V of applied voltage.

Chapter 5: Electron Transfer Pathways for Methane Production in Bioelectrochemical Anaerobic Digestion

5.1 Introduction

As well known, the anaerobic digestion process is still unstable and the organic matter reduction and methane production are not satisfactory (Song et al., 2004; Mata-Alvarez et al., 2014; Mao et al., 2015). The methane content in biogas is too low to use directly as a public fuel (Appels et al., 2008; Zhao et al., 2016). These limitations of anaerobic digestion are mainly caused by the inefficient anaerobic metabolic reactions, and the imbalance between the reaction steps (Mao et al., 2015; Feng & Song, 2016a; Shen et al., 2016). The anaerobic metabolic reactions of organic matter are commonly described as follows: organic matter is decomposed into monomeric organic substances by hydrolytic bacteria (Shrestha et al., 2014; Mir et al., 2016). In the case of complex organic wastes, the hydrolysis is often considered as a rate limiting step that controls all of the anaerobic metabolic reactions (Shin & Song, 1995, Khalid et al., 2011; Zhao et al., 2016). The monomeric substances are fermented by acidogenic bacteria to form hydrogen, formate, carbon dioxide, and small organic molecules such as lactate, succinate, fatty acids, and acetate (Shrestha et al., 2014; Mir et al., 2016; Zhao et al., 2016). Syntrophic acetogenic bacteria convert the small organic molecules into acetate by transferring the electrons to hydrogen and formate or releasing the electrons for direct electrical connection (Appels et al., 2008; Shrestha et al., 2014; Mir et al., 2016; Shen et al., 2016; Zhao et al., 2016). Methanogenic bacteria use acetate to produce methane, or use the electrons formed from hydrogen and formate or released from syntrophic bacteria to reduce carbon dioxide to methane (Lyberatos et al, 1999; Shrestha et al., 2014; Kouzuma et al., 2015; Feng & Song, 2016a; Zhao et al., 2016). However, in the anaerobic reaction steps, the metabolic rate differs depending on the bacterial groups. For example, the metabolic rate of methanogenic bacteria is

considerably slow and susceptible to the changes in environmental conditions such as organic loading rate, pH and temperature (Mao et al., 2015; Feng & Song, 2016a; Feng et al., 2016a; Song et al., 2016). Therefore, the balance in the metabolic rates of acidogenic/syntrophic bacteria and methanogenic bacteria could be destroyed easily by small external shocks. A new anaerobic metabolic reaction which is deliver the electron more efficiently and stably between the bacterial groups is great help to overcome these limitations of anaerobic digestion. In recent reports, the electroactive bacteria can transfer the electron directly to the methanogenic bacteria during the anaerobic fermentation of organic matter (Dube & Guiot, 2015; Kouzuma et al., 2015; Shen et al., 2016; Feng and Song, 2016a,b; Feng et al., 2016a; Song et al., 2016; Zhao et al., 2016). The features of methane production are highly dependent on the electron transfer characteristics of the anaerobic metabolic reactions. On the other hand, it has been revealed that the anaerobic digester equipped with the electrode with applied voltage, referred to as the bioelectrochemical anaerobic reactor, helps to enrich the electroactive bacteria (Lovley, 2011; Kouzuma et al., 2015; Dube & Guiot 2015; Zhao et al., 2016; Feng and Song, 2016a; Song et al., 2016). The methane production can be greatly enhanced by the electroactive bacteria in bioelectrochemical anaerobic reactors (Dube & Guiot, 2015; Feng and Song, 2016a,b; Song et al., 2016). However, the information on the electron transfer characteristics of the anaerobic metabolic reactions is still insufficient, and the influence of electroactive bacteria on the electron transfer pathways for methane production has also been largely unexplored.

In this study, the features of bioelectrochemical methane production were studied and compared with conventional anaerobic digestion in terms of electron transfer. The influence of the planktonic bacteria in the bulk solution on the electron transfer pathways were also investigated in a bioelectrochemical anaerobic batch experiment.

5.2 Materials and methods

5.2.1 Experimental set-up and its operation

For the bioelectrochemical anaerobic batch experiments, a cylindrical reactor (diameter: 10cm, height: 18 cm, effective volume: 1.0L) made of acrylic resin was used (Fig. 5.1). A separator and electrode assembly (SEA) was prepared by stacking in order of anode, separator and cathode, and then it was rolled into a cylindrical shape (diameter: 5 cm., height: 8 cm). The graphite fiber fabric (GFF) was used as the anode and cathode after modifying with multiwall carbon nanotube (MWCNT) and Ni to improve the electric conductivity, and submerging into a surfactant solution according to previous studies (Song et al., 2014; Feng et al, 2016a). A polypropylene nonwoven sheet was used as the separator between the anode and cathode. The SEA was installed at the center of the batch reactor over 5 cm from the bottom, and the anode and cathode were connected with a conductive titanium wire to an external DC power source. For the batch experiment, an anaerobic seed sludge (0.4L) and a medium (0.6L) were added into the reactor. The seed sludge was collected from an anaerobic digester for sewage sludge (S sewage treatment plant, Busan, South Korea). According to a previous work (Feng & Song, 2016a,b), the culture medium was prepared, and the initial concentrations in the batch reactors are 3 g/L of glucose, 2.45 g/L of NaH_2PO_4, 4.58 g/L of Na_2HPO_4, 0.31 g/L of NH_4Cl, 0.31 g/L of KCl, 10 mL/L of vitamins, and 5 mL/L of trace minerals. The initial VSS was 4,650 mg/L and the pH was 7.2. The prepared batch reactor was covered with an upper plate for air sealing and then flushed with nitrogen gas. A gas outlet, a gas sampling port, and a reference electrode inlet were installed on the upper plate of the batch reactor. The gas outlet of the upper plate connected to a floating gas collector using a rubber tube. The gas sampling port was covered with an n-butyl rubber stopper, and the bottom of the reference electrode inlet was attached with a sealing tube that immersed in the liquid phase in the reactor.

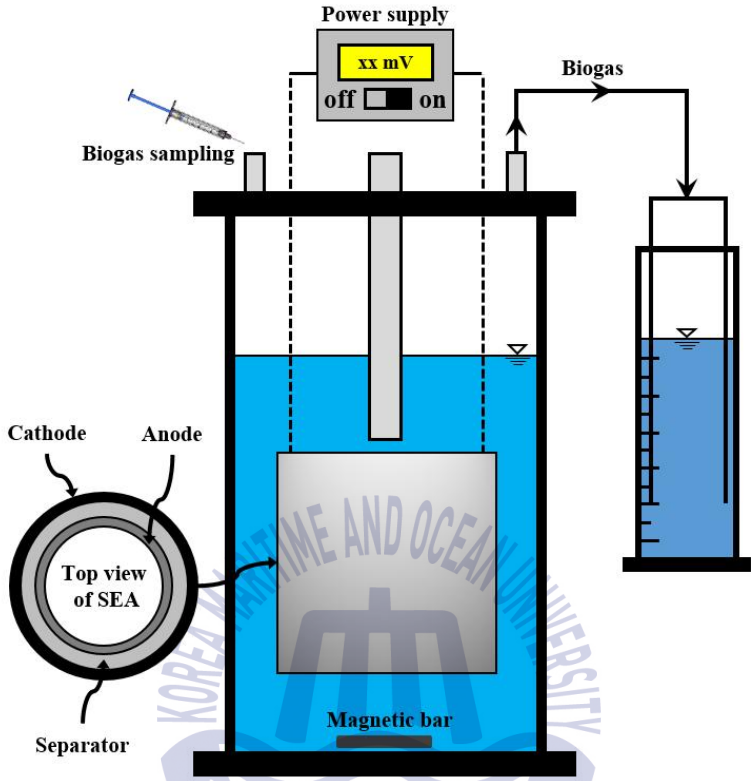


Fig. 5.1. Schematic diagram of bioelectrochemical anaerobic batch reactor.

Four batch reactors prepared for quadruple experiments in a same condition were installed in a constant temperature room of 35 ℃, and the medium was stirred with a magnetic bar. The operation of the batch reactor was started by applying a voltage of 0.5 V between the anode and cathode using an external DC power (OPM series, ODA Technologies Co., Incheon, South Korea). One more batch reactor prepared by the same method was used as a control, which was operated in a short circuit condition without the voltage application. During the operation, biogas production was monitored from the batch reactors. The anaerobic sludge in the batch reactor was settled down when the biogas production was not observed, and the supernatant liquid was replaced with a fresh medium. After two batch cycle operations for the

enrichment of electroactive bacteria, the bulk solution was taken from all of the batch reactors. The bulk solution were well mixed and allowed to settle for 30 min in a container. The settled sludge of 100 mL, 200 mL and 400 mL were put into the three batch reactors, respectively, and the substrate media were also filled up to the batch reactors. A batch reactor that was filled with the substrate medium only without the addition of the settled sludge was also prepared separately. The batch reactors are referred below as PAB0, PAB100, PAB200 and PAB400, respectively, according to 0 mL, 100 mL, 200 mL and 400 mL of the added sludge amounts. The initial VSS concentrations for PAB0, PAB100, PAB200 and PAB400 were 0 mg/L, 1,140 mg/L, 2,200 mg/L and 4,400 mg/L, respectively.

5.2.2 Analysis and calculation

During the operation of the batch anaerobic reactors, the biogas production over time was monitored using the floating type gas collector, and the biogas composition was analyzed using a GC (Gaw-Mac Instrument Co., PA, USA) with Porapak-Q column (6 ft×1/8th ” SS) and thermal conductivity detector. The temperatures of the inlet, oven, and detector of the GC were 50, 90, and 80°C, respectively. The production of the biogas including methane and hydrogen (Vbc,i) at each monitoring time interval was calculated from the measurements of the biogas volume and the biogas content (methane or hydrogen) data in the headspace of the batch reactor and the gas collector using the following mass balance equation (equation 3.3) (Feng & Song, 2016a,b). The biogas production was expressed as a standard temperature pressure (STP) state using the equation 3.2. The cumulative biogas production was determined by curve fitting to the modified Gompertz equation of equation 3.4, and the parameter including lag period, maximum biogas production rate and ultimate biogas production were obtained using the curve fitting toolbox of Matlab R2015b (Feng & Song, 2016a,b). Methane and hydrogen yields were calculated by dividing the amount of the methane and hydrogen produced by the COD grams removed during the batch experiment.

10 mL of liquid sample was also collected intermittently with a syringe to monitor the pH change, and filled to the reactor again. The electric current between the anode and cathode were monitored using a DMM (Keithley model 2700, Tektronic, Inc.), and the electrode potential of anode and cathode was measured with a portable digital multimeter (Fluke 87-V, Fluke Co., USA) and Ag/AgCl reference electrode (AlsCo., Ltd., Japan). According to the standard method, COD and VSS were measured at the initial and end of the batch experiment. The electron conversion efficiencies that are recovered as methane (MER, %) or hydrogen (HER, %) from the substrate were calculated by dividing the amounts of methane or hydrogen by the gram COD removed. The amount of the methane or hydrogen produced through the eDIET was estimated from the electric current, as equation 5.1.

Equation 5.1: $$P_c(mL) = \frac{\int_0^t idt}{nF} \times VPM$$

Where, i is the measured current (A), t is the time of the batch experiment, n is the number of electrons per mol of the methane (n=8) or hydrogen (n=2), F is the Faradays constant (96485 C/mol), VPM is the 22400 mL/mole of methane at standard temperature and pressure. The electron recovery efficiency (eER, %) for the forms of methane or hydrogen through the eDIET was obtained by dividing the Pe by the gram COD removed.

At non turnover condition before replacing the substrate medium, the electrochemical impedance spectra (EIS) for the anode and cathode were obtained in the frequency band ranging from 100 kHz to 10 MHz with an AC signal amplitude of 25 mV using an electrochemical instrument (ZIVE SP1, WonA Tech, South Korea) according to previous study (Feng & Song, 2016b). At open circuit condition, a working electrode and the other electrode as count electrode were connected to the terminals of electrochemical instrument, and an Ag/AgCl electrode was used as a reference electrode. The EIS data were fitted to a Randle type model that is mixed kinetic and diffusion control model using 'SMART Manager' analysis software. The

model includes a solution resistance in series with a double-layer capacitor, which is in parallel with the faradic reaction impedance consisting of a charge transfer resistance and Warburg element in series (Feng & Song, 2016b). In addition, cyclic voltammetry (CV) for the bulk solution (100mL) at non turnover condition was also conducted in the potential range between -1.0 and 1.0 V (vs. Ag/AgCl reference electrode) with a 10mV s-1 scan rate using the electrochemical instrument (ZIVE SP1, WonA Tech, South Korea). For the CV test, small pieces of stainless mesh (1 cm×1 cm) were used as the working and counter electrodes. The peak currents for the oxidation and reduction and the potential values at peak current were obtained from CV data using the 'SMART Manager' analysis software. The microbial communities of planktonic anaerobic bacteria in the bulk solution of PAB100 and PAB400, including archaea and bacteria, was analyzed at the end of experiment by pyrosequencing method according to a previous study (Feng et al., 2016a).

5.3 Results and discussion

5.3.1 Bioelectrochemical methane production

In the First batch experiment, the methane production increased sharply after the initial lag time of around 6 days in the bioelectrochemical anaerobic batch reactor (Fig. 5.2). The cumulative methane production in the bioelectrochemical reactor reached 904.8±39.6 mL, which was significantly higher than 583.6 mL of the control. In anaerobic digestion, the methane is one of the final products of the anaerobic fermentation process, which is produced through various electron transfer pathways (Lovley, 2011; Shrestha et al., 2014; Shen et al., 2016). The electron transfer for methane production differs in the transfer rate and efficiency depending on the electron transfer pathways. The maximum methane production rate in the bioelectrochemical reactor was 163.9 mL.d, which also higher than the control (Fig. 5.2). These features of methane production in the bioelectrochemical reactor indicate that

the electron transfer pathway for methane production was changed to the other efficient pathways from the conventional pathways in the control. In conventional anaerobic digestion, the methane is mainly produced through the indirect interspecies electron transfers (IIETs). In the IIETs, the electron transfer for methane production are mediated by the intermediates of anaerobic fermentation (), such as acetate, hydrogen/formate (Rotaru et al., 2014b; Shrestha et al., 2014; Kouzuma et al., 2015; Shen, et al., 2016), or sometimes mediated by the electron transferring redox shuttles (s) (Lovely, 2011; Richter et al., 2012; Shrestha et al., 2014; Dube & Guiot, 2015; Zhao, et al., 2016). On the other hand, in the bioelectrochemical reactors equipped with electrodes, the electroactive bacteria such as *Geobacter* and *Shewanella* species could be enriched (Kato, 2015; Shen et al., 2016; Zhao et al., 2016). These electroactive bacteria can directly transfer the electrons to the outside acceptor through C type cytochrome of the outer membrane or conductive pili (Rotaru et al., 2014b; Shrestha et al., 2014; Shen et al., 2016). Therefore, in an anaerobic reactor enriched with electroactive bacteria, the methane could be produced by the biological direct interspecies electron transfer (bDIET) between the electroactive bacteria and the methanogenic bacteria such as *Methanosaeta* and *Methanosarcina* species (Rotaru et al., 201a,b). The methane could sometimes be also produced by the direct interspecies electron transfer through electrodes (eDIET) or conductive materials (cDIET) (Rotaru et al., 2014a; Dube & Guiot, 2015; Shen et al., 2016; Shrestha et al., 2014). It seems that the electroactive bacteria in the bioelectrochemical reactor shifted the main electron transfer pathway into the DIET, which improved the methane production in the amount and production rate. This indicates that the DIET pathways has a higher electron transfer rate as well as higher electron transfer efficiency than the IIETs in the control.

In the second batch experiment, the methane production from the bioelectrochemical reactor was observed as soon as replacing the substrate depleted medium with fresh one. The quick response on the substrate is an important physiological characteristic of starved electroactive bacteria that was

observed in bioelectrochemical cells (Song, et al., 2015; Feng, 2016a,b). In the case of the control, the lag phase time for methane production was slightly longer than the bioelectrochemical reactor (Table 5.1). However, there are no significant differences in methane production features between the replicates of the bioelectrochemical reactors, compared to the first batch experiment (Fig. 5.2). These implied that the electroactive bacteria were well enriched and saturated in all replicates of the bioelectrochemical reactor. In the bioelectrochemical reactor, the maximum methane production rate that was estimated from the modified Gompertz equation was 267.2 mL.d, which was higher than in the first batch (Table 5.1). This suggests that the amount of electroactive bacteria was more enriched than the first batch operation; thereby the electron transfer pathway was considered to be more shifted to the DIETs from the IIETs. The ultimate methane production in the bioelectrochemical reactor was about 936.9 mL, which was considerably higher than 563.1 mL in the control. The methane production that improved greatly in the bioelectrochemical anaerobic reactor is in well agreement with the previous studies (Feng et al., 2016a,b; Song et al., 2016). In anaerobic digestion, the methane yield that indicates the electron transfer efficiency from organic substrate to methane is a useful parameter for the evaluation of the process performance. It seems that the electron transfer efficiency depends on the electron transfer pathway for methane production (Shen et al., 2016; Dube & Guiot, 2015; Zhao, et al., 2016). The methane productions through the IIET pathways involve the multiple enzyme reaction steps with electron losses (Shen et al., 2016). Furthermore, some parts of hydrogen as an electron transfer carrier may migrate to the gaseous phase due to its low solubility in the liquid. In the case of the other intermediates such as formate and acetate, it is not possible to transfer 100% of the electrons to methanogenic bacteria, and some can be left in the medium after the anaerobic digestion. Thereby, the pathways have some losses in the methane production from substrate, and resulting in a less methane yield. In the case of sIIET, such as humic substances, sulfur compounds, flavin-based compounds, cystein, etc., which serve as electron transfer shuttles, are only available in special condition that

are supplied or endogenously secreted in the anaerobic digester (Richter et al., 2012; Shrestha et al., 2014; Dube & Guiot, 2015; Shen et al., 2016). Furthermore, the reduced forms of the shuttles combined with electrons are also less likely to transfer 100% electrons to methanogenic bacteria in the anaerobic digester. The methane yield of the bioelectrochemical anaerobic reactor was 300.1 mL/g COD_r, which was higher than the control of 188.8/g COD_r. This suggests that the major electron transfer pathway in the bioelectrochemical reactor was the DIETs, which have higher electron transfer efficiency than the IIETs. On the other hand, the bioelectrochemical methane yield was less than the theoretical value of 350mL/g COD_r for glucose. It implies that the IIET pathways were still existing in the bioelectrochemical reactor as a appurtenant electron transfer pathway, and possibly the types of DIETs have some differences in the electron transfer rate and efficiency. In the case of the eDIET, the more positive anode potential and the more negative cathode potential than the redox potentials of the substances improve the directional oxidation and reduction rates at the electrodes respectively (Zhao et al., 2016). However, the methane production through the eDIET pathway may has some losses of the electrons in the transfer route due to the internal resistances, such as the ohmic, activation and polarization resistance of the bioelectrochemical device, as well as the IIET pathways (Song et al., 2010; Song et al., 2015a). Therefore, the methane yield that is lower than the theoretical value indicates that some of the methane production was produced by the IIETs or the eDIET pathways with low electron transfer efficiency. It is known that the electron transfer rate is high for the cDIET pathway (Kouzuma et al., 2015; Pereira et al., 2016), but it seems that the driving force for the electron transfer from the donor to the acceptor is weak. In this study, however, there was no conductive material to be considered in the medium used in the batch experiments.

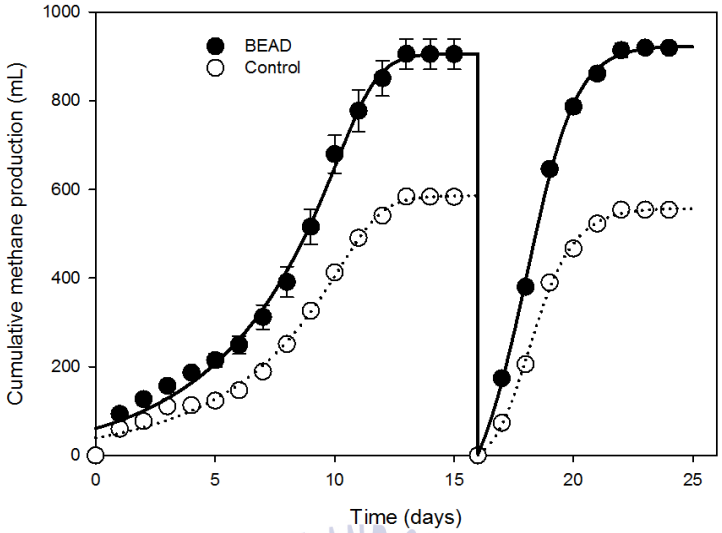


Fig. 5.2. Cumulative methane production in batch bioelectrochemical anaerobic reactor.

Table 5.1 Properties of biogas production in the bioelectrochemical anaerobic batch reactor.

Biogas	Parameter	BES	Control	PAB0	PAB100	PAB200	PAB400
Methene	P_u(mL)	936.9	563.1	268.9	694.1	976.9	977.8
	λ (d)	0.49	0.75	0.39	0.59	0.71	0.47
	um(mL.d)	267.2	175.4	62.3	136.6	203.4	207.1
	Adj-R^2	0.998	0.998	0.993	0.989	0.988	0.989
	Y(mL/g COD_r)	-	188.8	103.6	232.4	307.6	290.1
	MER(%)	-	53.9	29.6	66.4	87.9	82.9
Hydrogen	P_u(mL)	-	-	314.7	89.7	13.4	11.8
	Y(mL/g COD_r)	-	-	123.4	34.8	4.7	3.8
	HER(%)	-	-	8.8	2.5	0.3	0.3
eDIET pathway	P_e(mL)	-	-	259.3	170.4	155.3	72.1
	eDIET(%)	-	-	101.6	27.4	17.8	8.1
	eER(%)	-	-	30.0	18.2	15.6	2.3

(MER: electron recovery as methane, HER: electron recovery as hydrogen. Pe: biogas production as methane estimated from the electric current, eER: electron recovery through the eDIET)

5.3.2 Influence of planktonic bacteria in the bulk solution for the electron transfer

The biogas production in the bioelectrochemical reactor was considerably dependent on the concentrations of the planktonic bacteria in the bulk solution. The cumulative methane production was slowly increased after starting up the incubation of PAB0 (Fig. 5.3), and the ultimate methane production was 268.9 mL, which was approximately half amount of the control (Table 5.1). In the PAB0, there were no the planktonic bacteria in the bulk solution, but the electroactive bacteria that ferment organic matter and the methanogenic bacteria as their partners for electron transfer were well enriched on the anode and cathode, respectively (Fig. 5.2). This indicates that the main pathway transferring electrons for methane production in PAB0 is the eDIET that use the anode and cathode for the electron transfer. However, in the PAB0, internal resistance can cause the transfer losses while electrons are being transferred through the eDIET (Song et al., 2010; Song et al., 2015a). The EIS results for the electrodes show that the eDIET was significantly influenced by the charge transfer resistance of the electrodes in the internal resistance components (Table 5.2). Moreover, the charge transfer resistance of the cathode was 23.7ohms, which was much higher than the anode (Fig. 5.4). This indicates that the methane production in PAB0 was governed by the charge transfer resistance on the cathode. Interestingly, the considerable amount of hydrogen was also produced in PAB0, and the ultimate hydrogen production was amount to 314.7 mL. In anaerobic fermentation process, the electrons are generated from the anaerobic oxidation of low molecular organic or organic fatty acids (Lyberatos & Skiadas, 1999; Shrestha et al., 2014). This electron is transferred to the methanogenic bacteria through the IIET or DIET pathways, and the methanogenic bacteria produce methane by reducing the carbon dioxide (Shrestha et al., 2014). However, the slower carbon dioxide reduction rate than the electron generation rate can increase the $NADH/NAD^+$ ratio inside the fermentation bacteria (Nanqi et al., 2002; Dube & Guiot, 2015).

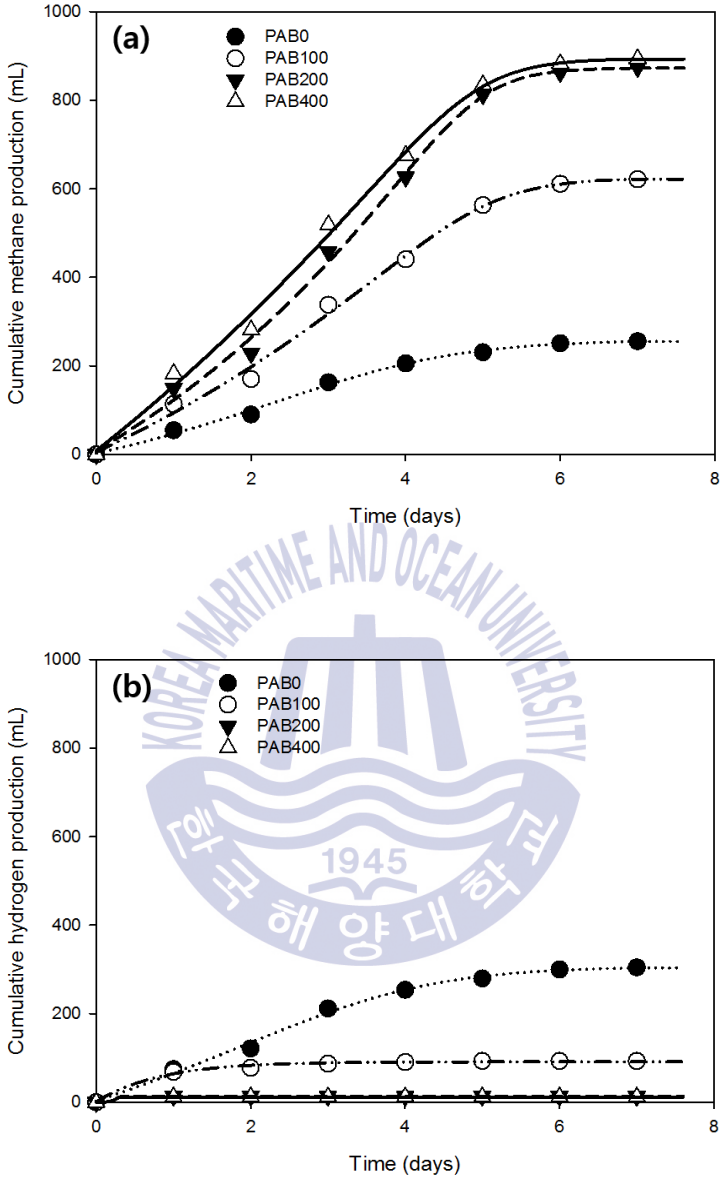


Fig. 5.3. Cumulative methane (a) and hydrogen (b) productions in the batch bioelectrochemical reactors with different planktonic bacteria concentrations.

It seems that the hydrogen production in the PAB0 was caused by relatively higher charge transfer resistance of the cathode than the anode. The methane yield in the PAB0 was quite small as 103.6 mL/g COD_r (Table 5.1). However, the hydrogen yield was 123.4 mL/g COD_r, which is equivalent to 30.9 mL/g COD_r of methane yield. This indicates that the electron recovery efficiencies in the PAB0 were 29.6% (MER, %=103.6×100/350) for methane and 8.8% (HER, %=30.9×100/350.0) for hydrogen, respectively. On the other hand, the percentage of the electron recovery (eER, %) that is estimated from the electric current between the anode and cathode was 30.0%. This means that the biogas production in the PAB0 cannot be explained by only the electrons recovered through the electrode, and there are possibly some other pathways rather than the eDIET for the methane and hydrogen production in the PAB0.

The CV result for the bulk solution in non-turnover condition prior to replacing the medium showed a peak current pair for the oxidation (Pa) and reduction (Pc) near 0.0V and -0.32V, respectively (Fig. 5.5). In this study, there were no artificially added electron shuttles or conductive materials in the medium used. It is possibly that there is a redox shuttle that mediates the electron transfer for oxidation and reduction near the formal potential of -0.16V. The shuttle is likely to be the substances, such as flavin-based compounds, that secreted endogenously by electroactive bacteria (Marsili et al., 2008; van der Zee et al., 2009; Wu et al., 2013; Shen et al., 2016). This means that some part of the hydrogen or methane is originated from the sIIET via the endogenously secreted shuttle.

Interestingly, the bioelectrochemical methane production was significantly improved by the presence of the planktonic bacteria in the bulk solution (Fig. 5.3a). In the PAB100, the maximum methane production rate was 136.6 mL.d, which was considerably higher than the PAB0 (Table 5.1). The ultimate methane production and the yield were 694.1 mL and 232.4 mL/g COD_r in the PAB100, respectively, which were also higher than the PAB0. This implies that the other electron transfer pathways rather than the eDIET

for methane production were activated in the PAB100 by the planktonic bacteria. However, the hydrogen production was significantly decreased (Fig. 5.3b), and the yield was only 34.8 mL/g COD_r. In the PAB100, the electron conversion efficiencies were 66.4% (=232.4×100/350) in methane, and 2.5% (=34.8/4×100/350) in hydrogen, respectively. In the EIS results, the charge transfer resistances for the anode and cathode in the PAB100 were 6.0 and 12.4 ohms, respectively, and the imbalance in the electron transfer rates for the eDIET between the anode and cathode was slightly improved compared to the PAB0 (Table 5.2). This indicates that the electrons generated from the fermentation process are more efficiently transferred to methanogenic bacteria through the other electron transfer pathways rather than the eDIET, and thus the $NADH/NAD^+$ ratio inside the fermentation bacteria was lower than that of the PAB0. The electron transfer efficiency through the eDIET in the PAB100 was only 18.2%, which was lower than the PAB0 (Table 5.1). These results suggest that the electron transfer pathway in the PAB100 is superior in the electron transfer rate and the efficiency than the PAB0. The electron transfer efficiencies from substrate to methane through the sIIET, bDIET and cDIET are considered to be better than the iIIET. In the CV results of the solution at a non turnover condition, the redox peaks in the PAB100 were observed at -0.08 V and -0.35 V, respectively (Fig. 5.5). However, the peak current heights for the oxidation and reduction were 1.61 mA and 1.39 mA, respectively, which were slightly higher than those of the PAB0 (Table 5.2). However, the peak current values are not significant enough to account for the increased electron transfer efficiency in the PAB100. In addition, there was no conductive substance to mediate the cDIET in the medium used in this experiment. Thus, it seems that the significant increase in the electron conversion efficiency in the PAB100 is mainly attributed to the bDIET between the electroactive bacteria and methanogenic bacteria present in the bulk solution. In previous studies, the active bDIET for methane production was observed in microbial aggregates in UASB reactors (Dube & Guiot, 2015). It suggests that the bDIET is also occurring between the electroactive bacteria and methanogenic bacteria that

are the suspended in the bulk solution.

The maximum methane production rate in the PAB200 with more the planktonic bacteria was faster than the PAB100 (Fig. 5.3). The ultimate methane production was 976.9 mL, which was much higher than the PAB100 (Table 5.1). The methane production through the eDIET that was estimated from the electric current was 155.3 mL, which was lower than the PAB100. Also, the hydrogen production was much smaller than the PAB100. In the CV data for the solution under non-turnover conditions, the redox peak potentials were observed at -0.05 V and -0.36 V, respectively. The peak current heights were slightly bigger than the PAB100, but it was not significant (Fig. 5.4). This means that only a small fraction of the increase in methane production can be explained in PAB200 by an increase in sDIET activity. The methane yield in the PAB200 was 307.6 mL/g COD_r, which was considerably higher 232.4 mL/g COD_r of the PAB100. This demonstrates that as the planktonic bacteria in the bulk solution increases, the methane production through the bDIET having a high electron transfer efficiency mainly increases, thereby improving the methane yield.

Table 5.2 Electrochemical analysis data in the batch bioelectrochemical reactors with different planktonic bacteria concentrations

Contents	CV	E_p (V)	i_p (mA)	EIS	Rohm (Ω)	Rct (Ω)	C (mF)	W $1/(\Omega\sqrt{Hz})$
PAB0	Oxidation	-0.01	1.46	Anode	2.01	7.45	9.08	1.17
	Reduction	-0.32	1.38	Cathode	3.12	23.71	8.76	1.01
PAB100	Oxidation	-0.08	1.61	Anode	2.04	5.99	5.41	1.14
	Reduction	-0.35	1.39	Cathode	3.96	12.36	17.24	1.19
PAB200	Oxidation	-0.05	1.61	Anode	1.93	5.29	8.32	0.41
	Reduction	-0.36	1.43	Cathode	2.77	10.87	15.49	0.59
PAB400	Oxidation	-0.12	1.83	Anode	2.01	3.91	8.85	0.68
	Reduction	-0.41	1.69	Cathode	2.65	8.06	8.35	1.23

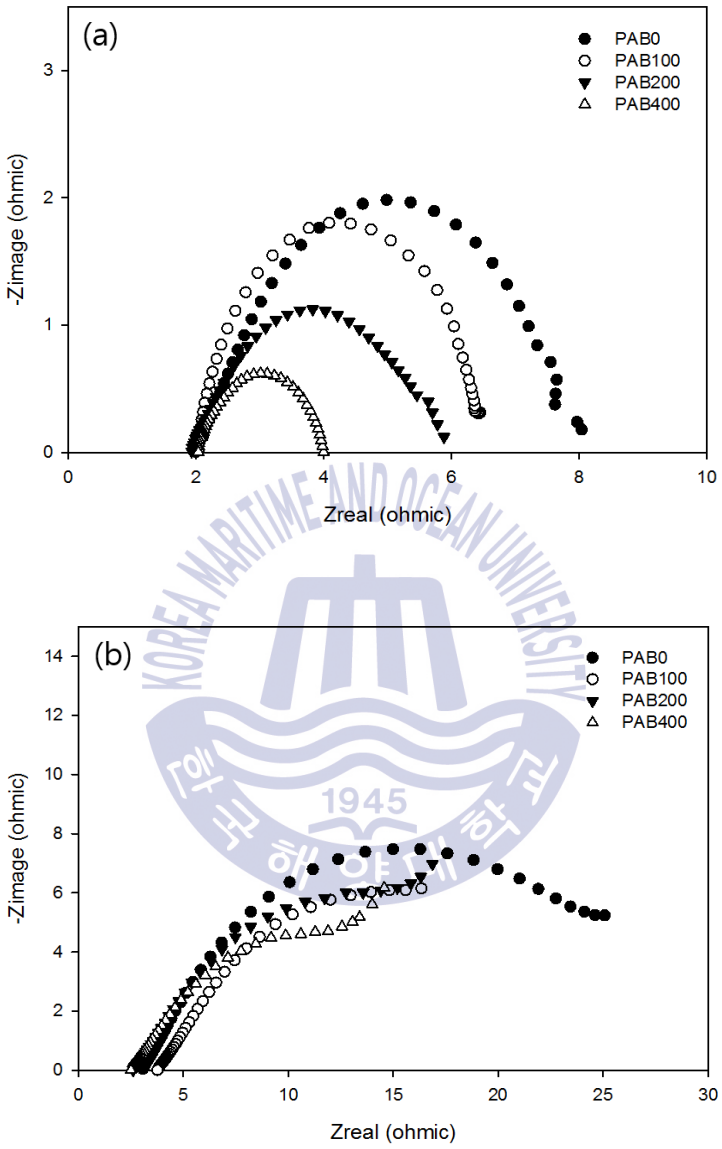


Fig. 5.4. EIS data for the anode (a) and cathode (b) in the batch bioelectrochemical reactors with different planktonic bacteria concentrations.

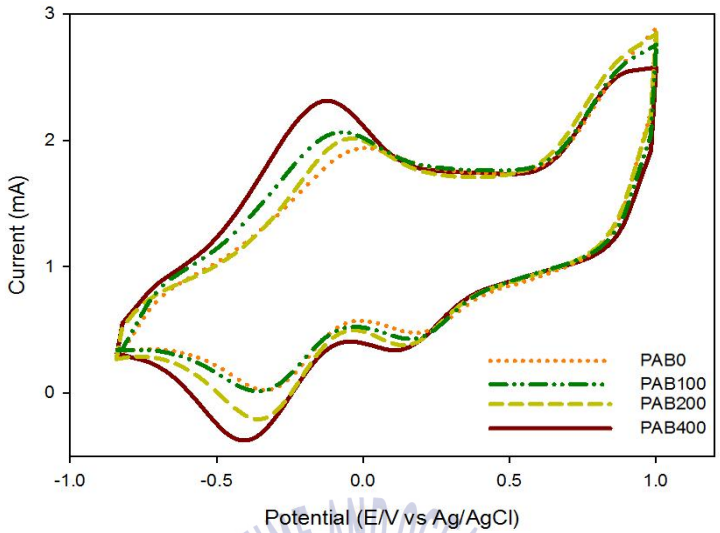


Fig. 5.5. Cyclic voltammogram for the liquid contents in the bioelectrochemical reactors with different planktonic bacteria concentrations at non-turnover condition.

In the PAB400, the features of methane production in ultimate methane production and maximum methane production rate were similar to the PAB200 (Fig. 5.3). However, the amount of methane produced from the electrode by the eDIET was 72.1 mL, which was approximately half of the PAB200, and the hydrogen production was also less than the PAB200. In the CV of the solution under the non-turnover condition, the redox peaks were observed at -0.12V and -0.41V, respectively, and the peak currents were slightly higher than those of the PAB200 (Fig. 5.5). This suggests that the portion of the sIIET slightly increases as the planktonic bacteria increases. The secretion of the electron shuttle compounds is usually observed under endogenous respiration (van der Zee et al., 2009). It seems that the limited available substrate in the PAB400 decreases the eDIET and the hydrogen production, and slightly increases the sIIET for methane production.

5.3.3 Microbial communities

The microbial communities in the planktonic bacteria of the PAB400 were clearly different from the PAB100 (Fig. 5.6). In the PAB100, Euryarchaeota (99.1%) were the most dominant phylum and the Bathyarchaeota content was only 0.8%. However, in the PAB400, the Euryarchaeota content slightly decreased to 97.9% and the Bathyarchaeota content (2.1%) slightly increased. Bathyarchaeota (2.1%) is a new archaeal lineage that requires acetoclastic methanogens in syntrophic association to remove acetate (Martin et al., 2016). It seems that the syntrophic association with acetoclastic methanogens in PAB400 was improved compared to that in the PAB100. At the class level, Methanomicrobia (78.3%), Methanobacteria (9.3%), Thermoplasmata (7.5%), and LNJC_c (2.9%) were dominant in the PAB100. Methanomicrobi and Methanobacteria belong to Euryarchaeota phylum, and were commonly found in paddy, lake, swamp, sediment, termite, and ruminant digestive or anaerobic digesters (Lueders et al., 2001). Thermoplasmata are eosinophils that survive in low pH environments. In the PAB400, the Methanomicrobia (78.6%) and Thermoplasmata (7.2%) contents did not significantly differ from those in the PAB100. However, the LNJC_c (3.3%), DHVE4b_c (2.4%), and MCG_c (2.1%) contents increased, while the Methanobacteria (6.2%) content slightly decreased. It is possible that LNJC_c, DHVE4b_c, and MCG_c, which increased in the PAB400, are the archeal classes that involve the DIET for methane production. At the species level, the Methanocorpusculum bavaricum (67.8%) that belong to the Methanomicrobia class was the richest species in the PAB100, followed by EU662692_s (5.7%), Methanobacterium congolense (5.1%), Methanosaeta concilii (4.7%), LNJC_s (2.6%), Methanocorpusculum parvum (2.5%) and Methanocorpusculum_uc (2.2%). In the PAB400, Methanosaeta concilii (8.8%), DHVE4b_c_uc_s (2.3%), and CU917078_s (1.8%) increased, but Methanocorpusculum bavaricum (62.6%) and Methanocorpusculum_uc (1.86%) decreased slightly. In the case of the Methanosaeta genus, such as Methanosaeta concilii, Methanosaeta harundinacea, Methanosaeta_uc, were found in both the PAB100 and PAB400.

However, the percentage of Methanosaeta species (10.2%) in the PAB400 was much higher than that (5.2%) in the PAB100. The Methanosaeta species are known to use electrons that are directly transferred from the electroactive bacteria (bDIET) to reduce carbon dioxide to methane, such as Geobacter species (Rotaru et al., 2014b; Shrestha & Rotaru, 2015; Shen et al., 2016; Zhao et al., 2016). It is possible that DHV4b_c_uc_s and CU917078_s, which increased in the PAB400 compared to the PAB100, are also involved in bDIET. However, Methanobacterium congolense and Methanocorpusculum_uc are greatly reduced in the PAB400 compared to the PAB100. Methanobacterium congolense is a hydrogenotrophic methanogens that uses hydrogen and carbon dioxide as the only substrates for methane production (Cuzin et al., 2001; Zhao et al., 1989). This indicates that the growth of the species involving the iIIET route using the intermediates such as hydrogen/formate for methane production was suppressed in the PAB400 compared to in the PAB100. It seems that the DIET pathways with high electron transfer efficiency are more competitive than the iIIET pathway with low electron transfer efficiency in the bioelectrochemical anaerobic reactor.

In the bacterial communities, the dominant phylum in the PAB100 was Firmicutes (45.4%) and Bacteroidetes (27.0%), followed by Proteobacteria (7.7%), Chloroflexi (5.1%), Verrucomicrobia (2.4%), Synergistetes (1.8%), Thermotogae (1.7%), Euryarchaeota (1.5%), and Actinobacteria (1.4%) (Fig. 5.7a). The dominant members of the bacterial phylum in the PAB400 were similar to those in the PAB100. However, while the abundance of Firmicutes (37.2%) and Bacteroidetes (20.5%) was significantly reduced, the other phylums including Proteobacteria (8.8%), Cloacamonas_p (6.5%), Chloroflexi (5.7%), Thermotogae (3.1%), Euryarchaeota (2.7%), Actinobacteria (2.7%), and Synergistetes (2.3%) were increased in the PAB400. In previous studies, these microbial phylum including Firmicutes, Proteobacteria, Chloroflexi, Actinobacteria, and Synergistetes were commonly observed using the bioelectrochemical apparatus (Vandecandelaere, et al., 2010; Blanchet et al., 2014; Wu et al., 2014b).

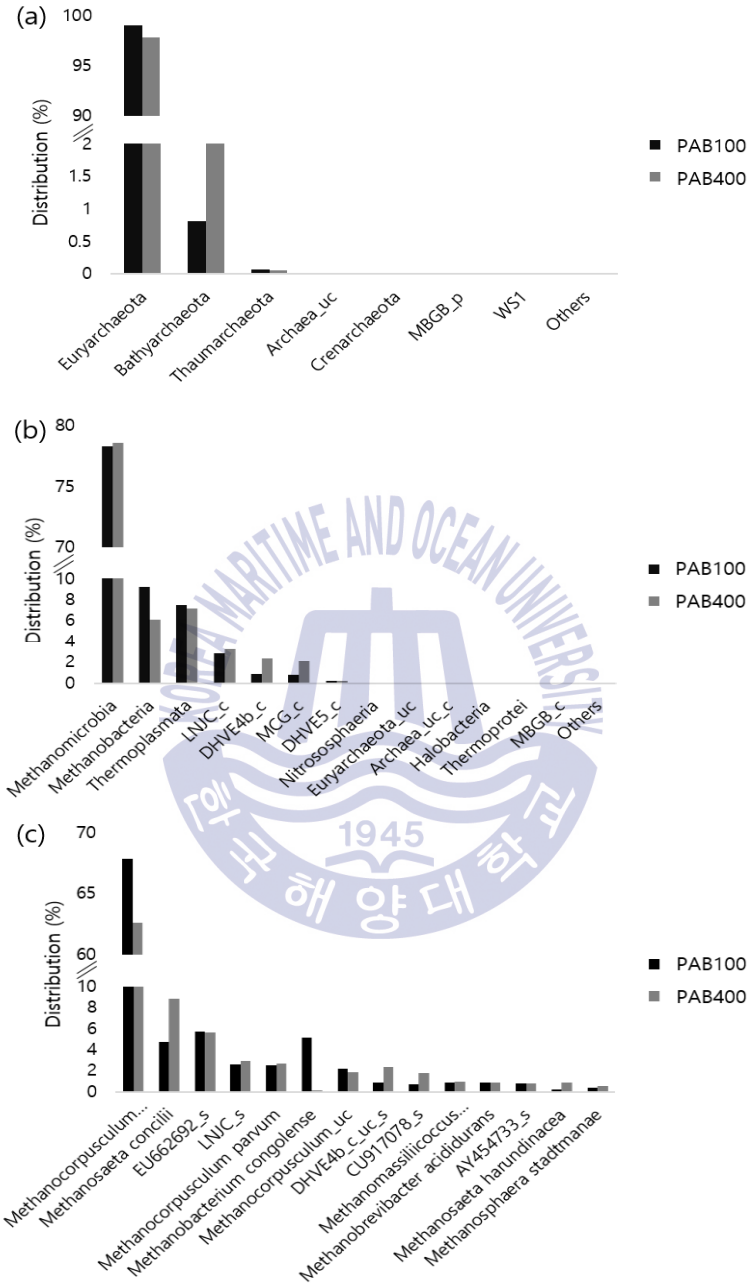


Fig. 5.6. Percentage of (a) phylum, (b) class and (c) species for planktonic archaea communities in the PAB100 and PAB400.

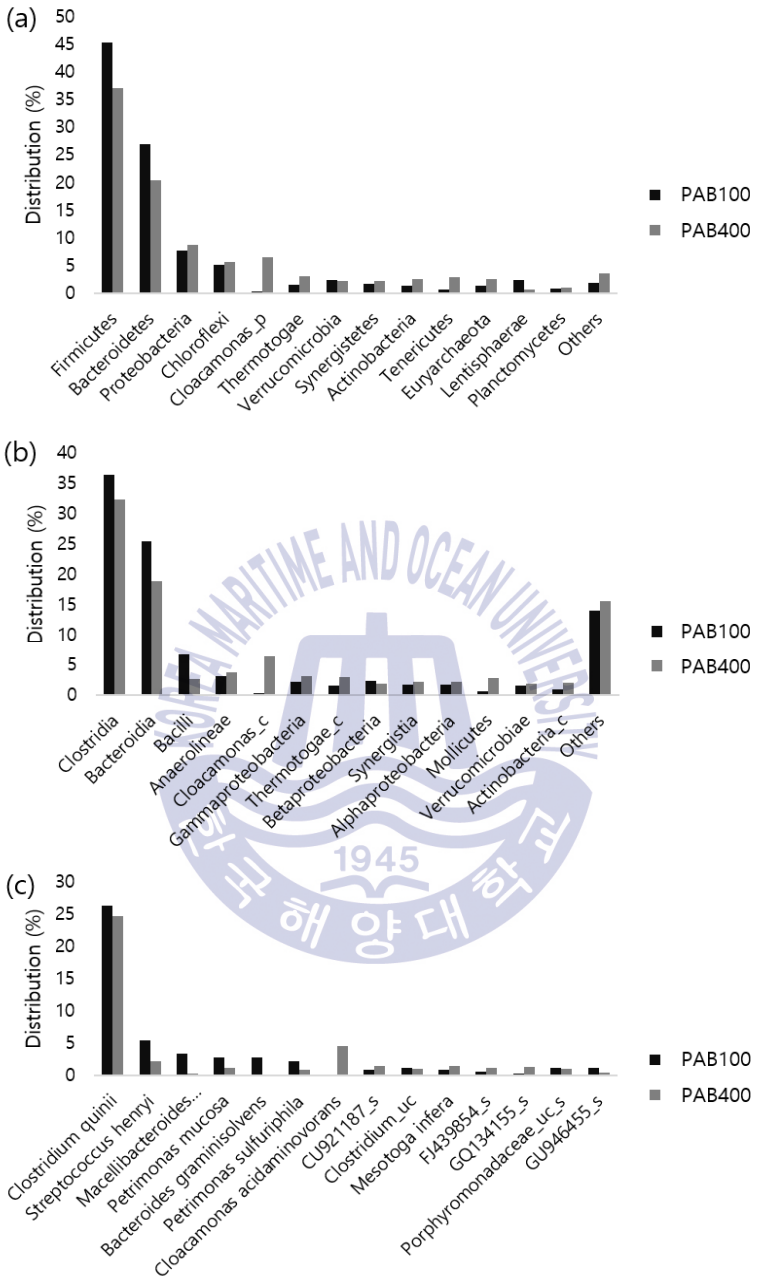


Fig. 5.7. Percentage of (a) phylum, (b) class and (c) species for planktonic bacteria communities in the PAB100 and PAB400.

While Cloacamonas is a phylum associated with the IIET, a link to the DIET has yet to be reported (Juste-Poinapen et al., 2015). It is possibly that the electroactive bacteria involved in the DIET pathways and syntrophic bacteria linked to the iIIET are likely to belong to the phylum increased in the PAB400. In the classes, Clostridia (36.1%), Bacteroidia (25.6%), Bacilli (6.8%), Anaerolineae (3.2%), Betaproteobacteria (2.5%), and Lentisphaeria (2.4%) were dominant in PAB100. In the PAB400, the portions of Cloacamonas_c (6.5%), Gammaproteobacteria (3.2%), Thermotogae_c (3.1%), Mollicutes (2.9%), Methanomicrobia (2.4%), Synergistia (2.3%), Alphaproteobacteria (2.2%), and Actinobacteria_c (2.1%) increased significantly (Fig. 5.7b). Deltaproteobacteria, which is known to consist mainly of the Geobacter species, was only 0.8% in PAB100 but increased to 1.3% in the PAB400. This reveals that the electroactive bacteria belong to the classes that increased more in the PAB400. The species were diverse in the PAB100, and the dominant species were Clostridium quinii (26.3%), Streptococcus henryi (5.5%), Macellibacteroides fermentans group (3.4%), Petrimonas mucosa (2.9%), Bacteroides graminisolvens (2.8%), and Petrimonas sulfuriphila (2.3%) (Fig. 5.7c). However, in the PAB400, portions of Cloacamonas acidaminovorans (4.6%) and CU921187_s (1.5%) were specifically increased while the species including Clostridium quinii (24.7%), Streptococcus henryi (2.3%), Macellibacteroides fermentans group (0.4%), Petrimonas mucosa (0.9%), and Bacteroides graminisolvens (0.2%) had reduced. It suggests that the increased species in PAB400 were involved in the enrichment of electroactive bacteria for increasing the bDIET pathway for methane production.

5.3.4 Implications of electron pathways for methane production

In anaerobic digestion, a better understanding of the pathway for the electrons transferring from substrate to methane is a very important strategy in improving anaerobic digestion performance. The electron transfer efficiency for methane production depends on the pathway for the electron flow.

Therefore, the maximum methane production rate and methane yield in an anaerobic digestion are governed by the electron transfer pathways. In conventional anaerobic digestion, the electrons that are generated from the fermentation process are mainly transferred indirectly to methane via the intermediates, such as acetate, hydrogen and formate (Shrestha et al., 2014; Mir et al., 2016; Zhao et al., 2016). Methane production via the indirect electron transfer pathway is achieved by a series of enzymatic reactions with electron transfer losses (Shen et al., 2016). In addition, hydrogen/formate can be produced only when the hydrogen partial pressure is lower than 1 bar, but when the hydrogen partial pressure is higher than the limit level, the ratio of $NADH/NAD^+$ becomes high, which eventually inhibits the acid fermentation that is generating electrons (Garrigues et al., 1997; Lyberatos & Skiadas, 1999; Reeve et al., 2015). The metabolic rate for methane production in conventional anaerobic digestion is very slow, and sensitive to the environmental condition (Song et al., 2016; Feng et al., 2016a). Therefore, acetate can be easily accumulated when the organic loading rate is high or the environmental condition is fluctuated for the anaerobic digestion (Song et al., 2004; Gomez et al., 2006; Mao et al., 2015;). When the hydrogen partial pressure and acetate concentration are increased up to over the limit values, the pH decreases and the metabolic reaction of the methanogens is inhibited, so that the anaerobic digestion processes can be destroyed (Thiele et al., 1988; Cazier et al., 2015).

Recently, it has been introduced that the role of electron shuttles (sIIET), which combine with electrons generated from the fermentation process to transfer electrons into methane, contribute to methane production in anaerobic digestion (Lovely, 2011; Richter et al., 2012; Shrestha et al., 2014;). These electron shuttle materials include humic substances, sulfur compounds, cysteine, etc., are often present in the nature condition, as well as flavin-based compounds which are endogenously secreted by bacteria (Richter et al., 2012; Shrestha et al., 2014; Dube & Guiot, 2015). The electron shuttles also play an important role for methane production at the special

conditions in which the shuttle materials coming from the outside or secreted endogenously by bacteria. Nowadays, the symbiotic bacteria was recognized that closely bound to the methanogens to transfer hydrogen or the electrons directly generated from fermentation to methanogens in the UASB granules (Dube & Guiot, 2015; Shen et al., 2016). The high efficiency of methane production in UASB process is considerably attributed to the DIET between the fermenting syntrophs and the methanogens (Kato, 2015; Zhao et al., 2016). Therefore, the DIET reaction is commonly recognized to have high efficiency in electron transfer for methane production. Generally, the fermentation bacteria involved in the DIET have been considered to be electroactive iron reducing bacteria, such as Shewanella and *Geobacter* species (Kato, 2015; Shen et al., 2016; Zhao et al., 2016). However, more species were discovered as electroactive bacteria and known to be involved in the DIET (Rotaru et al., 2014a,b; Dube & Guiot, 2015). It is well known that that electroactive bacteria directly transfer electrons (cDIET) to methanogens through the conductive substances, such as activated carbon, magnetite, iron oxide, and carbon nanotubes in anaerobic digestion (Pereira et al., 2016; Rotaru et al., 2014a). However, the cDIET through conductive materials is the passive methods for improving methane production as well as the bDIET in UASB granules.

On the other hand, the electroactive bacteria involved in the DIET for methane production are easily enrichment in a bioelectrochemical reactor equipped with anode and cathode (Shen et al., 2016; Zhao et al., 2016). There are several reports were published that the methane production is greatly enhanced in anaerobic digestion with bioelectrochemical devices enriched with electroactive bacteria (Lovely, 2011, Feng et al., 2016a, Zhao et al., 2016). In the eDIET, the electron is easily transferred to the anode with higher potential than the oxidation potential of the organic matter. The electron is directly moved from the anode to the cathode with lower potential than the reduction potential of carbon dioxide to methane. However, there are some losses in the electron transfer processes due to the internal resistance in

bioelectrochemical devices. In the anaerobic digestion equipped with bioelectrochemical devices, electroactive bacteria such as *Geobacter* are easily enrichment not only on the surface of electrode but also in the bulk solution, and they can transfer the electron (bDIET) directly to the acetoclastic mathanogens such as Methanosaeta and Methanosarcina, which is known to be the most efficient way to transfer mass amount of electrons with minimal loss (Kato, 2015; Shen et al., 2016; Zhao et al., 2016). The approaches involved in the eDIET and bDIET in bioelectrochemical reactors are an aggressive method for enhancing the methane production via the enrichment of electroactive microorganisms. In order to maximize the anaerobic digestion efficiency, it is necessary to reduce the methane production through the eDIET pathway and to selectively improve the bDIET in bioelectrochemical anaerobic digestion.

In this study, it revealed that the planktonic bacteria in the bulk solution have interest relationship with the methane production through the bDIET pathway. The methane production through the bDIET was the main pathway in the absence of the planktonic bacteria in the bulk solution. However, the sIIET that is mediated by the electron shuttle secreted endogenously by bacteria was also considered as a part of the methane production. In the absence of the planktonic bacteria, the methane yield was low as 103.6 mL/g COD_r, and a considerable amount of hydrogen was produced (Table 5.1). The methane production rate was quite slow, and the electron transfer efficiency was only 30%. From the EIS results, it confirmed that these results were ascribed to the large internal resistance of the bioelectrochemical device. However, when the planktonic bacteria were increased in the bulk solution, the cumulative hydrogen production decreased, and methane production rate and methane yield increased greatly. In addition, the amount of methane produced by the eDIET reaction, which was estimated from the electric current, also decreased. This means that the bDIET is considerably improved by the planktonic bacteria in the bulk solution. The decrease in eDIET of electrode is believed to be due to the increase of bDIET with higher electron

transfer efficiency in the bulk solution. When the concentration of the planktonic bacteria in the bulk solution was increased more, the hydrogen production decreased in proportion to the planktonic bacteria, and the methane production rate and the yield were further increased. As the methane production through the eDIET decreased, the methane yield gradually increased. However, the CV data showed that the sDIET also gradually increased. These results indicate that the bDIET and sIIET pathways have better electron transfer efficiency than the iIIET or the eDIET pathways. Thus, the best strategy of anaerobic digestion is to improve the bDIET via the enrichment of electroactive bacteria by coupling a bioelectrochemical device in anaerobic digester with abundant planktonic bacteria in the bulk solution.

5.4 Conclusions

The anaerobic digestion coupled with bioelectrochemical device reactor enriches electroactive bacteria in bulk solution, as well as the electrode surface. The bioelectrochemical reactor with abundant planktonic bacteria improves up to 60% in methane production rate and methane yield, compared to conventional anaerobic digestion. The electroactive bacteria in the bulk solution with more the planktonic bacteria improve the methane production more through the direct interspecies electron transfer biologically (bDIET), as well as via the endogenous electron shuttle (sIIET). However, the methane production through the electron transfer via the electrode (eDIET) has a relative large electron transfer losses due to the internal resistance of the bioelectrochemical device. In bioelectrochemical anaerobic digestion, the increases in the planktonic bacteria in the bulk solution is a good strategy to improve the methane production by improving the direct interspecies electron transfer biologically, as well as the electron transfer via the endogenous electron shuttle.

Chapter 6: Performance of Upflow Anaerobic Bioelectrochemical Digester for Acidic Distillery Wastewater Treatment

6.1 Introduction

Distillery wastewater is characterized by a low pH (3.5-4.5), high contents of organic matter, highly dissolved inorganic substances, and a dark brown color, and it causes serious pollution problems when discharged into the environment (Basu et al., 2015; Samsudeen et al., 2016). Commonly, the distillery wastewater has been treated by high-rate anaerobic digestion processes, such as upflow anaerobic sludge blanket (UASB), anaerobic filter and anaerobic fluidized bed reactor (Akarsubasi et al., 2006; Mohana et al., 2009; Pant & Adholeya, 2007). The organic matter contained in distillery wastewater could be removed at rates of 40-70% by an UASB process in previous studies (Harada et al., 1996; Souza et al., 1992). However, the low pH of distillation wastewater remains a source of many limitations of anaerobic digestion, such as unstable digestion, and process operations at a low organic load (OLR) (Pant & Adholeya, 2007). Anaerobic digestion of organic matter consists of several consecutive biochemical reactions, including hydrolysis, acidogenesis and methanogenesis (Song et al., 2004). The insoluble organic polymers contained in wastewater are initially broken down into soluble monomers during the hydrolysis process (Song et al., 2004; Mir et al., 2016), after which acidogenic bacteria ferment the soluble monomers into small organic molecules, such as fatty acids, carbon dioxide and ammonia, and generate electrons, which are used to form hydrogen and formate. Finally, the acetate is converted into methane by acetoclastic methanogens, and the carbon dioxide is also reduced to methane by hydrogenotrophic methanogens using the electrons from the hydrogen and formate (Mir et al., 2016; Shrestha et al., 2014). Generally, the optimum pH for the growth of acidogenic bacteria is ranged from 4.0 to 6.0 (Bengtsson et al., 2008), whereas the optimum pH for methanogenic bacteria is from 6.6 to

7.6 (Ritmann & McCarty, 2001; Yang et al., 2015). In particular, the activity of methanogenic bacteria is very susceptible to pH and temperature conditions (Pant & Adholeya, 2007). Therefore, the low pH of acidic distillery wastewater can inhibit the activity of methanogenic bacteria, and such cases require a neutralization process as a pretreatment (Shrestha et al., 2014; Kato et al., 2015).

On the other hand, it has been reported that bioelectrochemical anaerobic digestion with electroactive bacteria offers high performance capabilities in methane production as well as organic matter removal (Feng et al., 2016a; Song et al., 2016). Thus far, the high-performance capabilities of bioelectrochemical anaerobic digestion have been described mainly as stemming from the anodic oxidation of low molecular organic matter and the cathodic reduction of carbon dioxide (Villano et al., 2016; Wang et al., 2009). In a bioelectrochemical anaerobic reactor, electroactive bacteria growing on the anode surface ferment low molecular organic materials into carbon dioxide, protons and electrons. The electrons are transferred to the anode and moved to the cathode through a conductive external circuit by a potential difference between the electrodes, after which the carbon dioxide and protons are reduced to methane on the surface of the cathode (Gajaraj et al., 2017; Feng & Song, 2016b). However, it is difficult to describe fully the methane production process which takes place during bioelectrochemical anaerobic digestion only in terms of the reduction of carbon dioxide on the cathode surface (Feng & Song, 2016a,b). Recently, direct or proximity contact between syntrophs and methanogenic bacteria has been recognized as an important role in improving the performance of anaerobic digestion (Kato, 2015; Zhao et al., 2016). Methanogenic bacteria can accept electrons from syntrophs through direct cell contact with electroactive bacteria or conductive materials (Kato, 2015; Shen et al., 2016). Meanwhile, electroactive bacteria can easily be enriched in a bioelectrochemical anaerobic reactor, which is an anaerobic reactor coupled with a bioelectrochemical device equipped with an anode and cathode pair (Zhao et al., 2016; van Eerten-Jansen et al., 2015). It

is likely that electroactive bacteria that transfer electrons directly to methanogenic bacteria contribute significantly to the performance of bioelectrochemical anaerobic digestion (Song et al., 2016; Zhao et al., 2016). In previous studies, methane production during bioelectrochemical anaerobic digestion was shown to be relatively less affected by environmental factors such as the pH and temperature, but the mechanisms involved were not fully elucidated (Feng et al., 2016a; Yuan et al., 2011). It is likely that the bioelectrochemical pathways for methane production are less susceptible to the environmental conditions of the anaerobic reaction (Feng et al., 2016b). However, little is known about the effect of the pH on the electron transfer depending on the pathways for methane production during anaerobic digestion. Therefore, it is necessary to gain a better understanding of the effects of the pH on the electron transfer for methane production, especially with regard to the bioelectrochemical pathways through electroactive bacteria enriched during the bioelectrochemical anaerobic digestion process.

In this study, the influence of the pH on the electron transfer pathway was investigated in an upflow anaerobic bioelectrochemical reactor (UABE) using neutralized acidic distillery wastewater and compared to that in an UASB reactor. In addition, the influence of effluent recirculation on the electron transfer for methane production in both upflow anaerobic reactors was also studied. The UABE reactor with effluent recirculation was proposed for acidic wastewater treatment, as an alternative to high rate anaerobic process. The results of influent pH and effluent recirculation were published in ‘International Journal of Hyrogen Energy’ and ‘Bioresource Technolgoy’, respectively (Feng et al., 2017a,b).

6.2 Materials and methods

6.2.1 Electrode fabrication

For the anode and cathode, graphite fiber fabric (GFF, Samjung C&G Co., South Korea), multiwall carbon nanotube (MWCNT, Carbon Nano-material Technology Co., Ltd., South Korea), exfoliated graphite (EG, Hyundai Coma Industry, Inc., South Korea), and nickel dichloride were prepared (Feng & Song, 2016b). The GFF and MWCNT were immersed in concentrated nitric acid for 24 hours, and then rinsed with running tap water to remove impurities and improve the surface hydrophilicity. The EG was exfoliated by microwave radiation for 10 s, and then reduced in hydrazine solution, as in a previous study (Kim et al., 2015b). GFF was placed in an electrolyte solution in which 1 g of $NiCl_2$ was dissolved in 1 L DI water, and then a voltage of 30 V was applied for 10 min to uniformly deposit nickel on the GFF surface electrophoretically. Then, a mixture paste of MWCNT and EG was screen-printed on the surface of the GFF to form a scaffold layer, which was hot-pressed at 200 °C for 15 min. The paste was prepared by mixing 1 g of MWCNT, 1 g of EG, 0.1 g of $NiCl_2$, 10 mL of binder, and 20 mL of ethanol. The binder was obtained by dissolving 1 g of the coal tar pitch in 10 mL of toluene. Finally, the electrode was immersed in 1% sodium dodecyl sulfate (SDS) solution, to improve the hydrophilicity over the previous study (Feng & Song, 2016a).

6.2.2 Upflow anaerobic reactors (UABE, UASB) and operation

The upflow anaerobic reactors used in this experiment were prepared using cylindrical acrylic resin (effective volume 5.5L, inner diameter 15cm) (Fig. 6.1a) (Feng et al., 2016b). The bottom of each reactor was shaped as a cone to evenly distribute the influent wastewater inside of the reactor, and the upper end of the reactor in each case was ensured to be air-tight using a flange-type cover plate. An inlet valve was installed on the lower wall of each reactor, and in each case an outlet port was installed 15 cm below the cover plate. An L-shaped acrylic tube was connected to the inlet valve inside

the reactor to allow influent wastewater to flow downward to the cone-shaped bottom. The outlet port had a U-shaped tube so that the headspace of the reactor would be blocked from outside air.

A gas sampling port, a reference electrode holding port, and a biogas venting port were installed on the cover plate. The gas sampling port was covered with an n-butyl rubber stopper, and the bottom of the reference electrode holding port had an acrylic tube immersed in a liquid solution attached to maintain air-tightness. The biogas venting port was connected via a rubber tube to a floating-type collector. The inside of the gas collector was filled with acidified brine to prevent the dissolution of the biogas (Walker et al., 2009). The separator and electrode assembly (SEA) that was stacked with the anode, the polypropylene nonwoven fabrics and the cathode formed a spiral shape (Fig. 6.1b). These components were installed inside the upflow reactor to complete the UABE reactor, and another upflow reactor without a SEA was used as the UASB reactor.

The upflow anaerobic reactors were placed in a temperature-controlled room held at 35±2 ℃. The seed sludge was prepared by mixing granular sludge with anaerobic sewage sludge in halves by volume, and the upflow anaerobic reactors were each seeded with 3 L of this sludge. The granule sludge was collected from a commercial UASB reactor (HJ Ethanol, Jeonju, South Korea), and the anaerobic sewage sludge was obtained from an anaerobic digester for sewage sludge (S-STP, Busan, South Korea). The distillery wastewater collected from MH Ethanol (Masan, South Korea) was then continuously fed into the upflow anaerobic reactors through the inlet valves with a peristaltic pump. The characteristics of the granular sludge, the anaerobic sludge and the distillery wastewater are presented in Table 6.1.

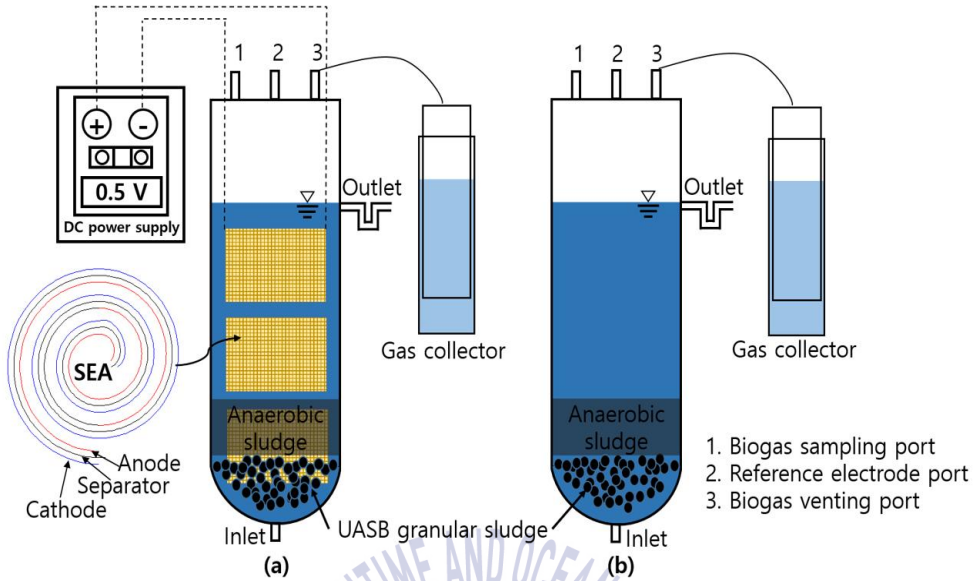


Fig. 6.1. Schematic diagram of (a) the upflow anaerobic bioelectrochemical reactor (UABE) and (b) the upflow anaerobic sludge blanket (UASB).

Table 6.1 Characteristics of the seed sludge and distillery wastewater

Parameters	Granular sludge	Anaerobic sludge	Distillery wastewater
pH	7.34	6.52	3.6±0.2
Alkalinity (mg/L as $CaCO_3$)	-	683	-
VFAs (mg/L as COD)	-	364	-
TCOD (g/L)	-	20.6	35.7±1.7
SCOD (g/L)	-	1.9	25.5±1.4
TS (g/L)	-	16.2	27.5±3.1
VS (g/L)	-	8.5	24.2±3.3
Sulfate (g/L)	-	-	1.5±0.1
Conductivity (mS/cm)	-	-	2.59±0.27

6.2.3 Influent pH in upflow anaerobic bioelectrochemical reactor

In the UABE reactor, a direct-current power supply (OPM series, ODA Technologies Co. Ltd., Inchoen, South Korea) was used to maintain the potential difference between the anode and cathode at 0.5 V. The upflow anaerobic reactors began at an OLR of 1.0 g COD/L.d and then increased to an OLR of 8g COD/L.d with a stepwise increase in the flowrate of the distillery wastewater at pH 3.6. The acidic distillery wastewater was neutralized to pH 5.6, 7.0 and 7.5 using sodium bicarbonate. When the upflow reactors reached a steady state, the influent wastewater was gradually replaced with additional neutralized wastewater. The steady states of the upflow anaerobic reactors were confirmed by methane production and organic matter removal and by the pH and alkalinity levels. The amount of sodium bicarbonate needed to neutralize the acidic distillery wastewater to pH 5.6 was 10±2 g/L. The neutralization of the wastewater to pH 7.0 and pH 7.5 required two and four times the amounts of sodium bicarbonate needed to neutralize to pH 5.6.

6.2.4 Effluent recirculation in upflow anaerobic bioelectrochemical reactor

The upflow reactors started at 1 g COD/L.d of organic loading rate (OLR), and were stepwise increased to 8 g COD/L.d. The steady state conditions were confirmed by state variables, such as pH, alkalinity, methane production and organic matter removal. In the effluent recirculation experiment, the ratio of the recirculated effluent to the influent was changed to 1.5, 1.0, 0.5, and 3.0.

6.2.5 Analysis and calculation

During the operation of the upflow anaerobic reactors, the effluent pH was checked daily with a pH meter (Orion Model 370). Intermittently, the sulfate and COD of the effluent were analyzed according to the standard method (2005), and the alkalinity and VFA concentration were measured with the

titration method (Song et al., 2016). The VFA composition was analyzed with a HPLC (Dionex Ultimate 3000, Germering, Germany) equipped with TCC-3000 thermostat column and a UV detector. The biogas was monitored daily and the biogas composition was analyzed by gas chromatography (Series 580, GOW-MAC Instrument Co., PA, USA) with a thermal conductivity detector and a Porapak Q column (6ft×1/8“ SS). The methane production amount was converted to the standard pressure and temperature (STP) state by the correction of the water vapor pressure at 35 ℃ (Feng & Song, 2016a). The methane produced from the electrode was calculated from the electric current between the anode and cathode according to a technique used in a previous study (Zhao et al., 2015), and the electric current was monitored using a digital multimeter (DMM: Ni cDAQ-9174, National Instruments). The electrochemical impedance spectroscopy (EIS) experiments were performed on the anode and cathode with an Ag/AgCl reference electrode using an electrochemical instrument (ZIVE SP1, WonATech, South Korea). The potential wave signal (25 mV) was applied to the electrode at frequencies ranging from 10 mHz to 10 kHz. The impedance responses were fitted using the ‘SMART Manager’ software into a Randle-type equivalent circuit model with mixed kinetic and diffusion control. The Randle-type equivalent circuit model includes the ohmic resistance of the solution in series with a double-layer capacitor, which is in parallel with the Faradaic reaction impedance consisting of the charge-transfer resistance and a Warburg element in series (Feng et al., 2016b). The energy efficiency of the UABE was estimated with the recovery energy of methane relative to the sum of the removed substrate energy and the consumed electric energy via Equation 4.1. The energy efficiency of UASB reactor was calculated with the same equation without electric energy.

6.3 Results and discussion

6.3.1 Influence of influent pH on upflow anaerobic bioelectrochemical reactor

① Features of methane production

The methane production features from the UABE and UASB reactors treating the acidic distillery wastewater were significantly affected by the influent pH (Fig. 6.2a). For the acidic influent of distillery wastewater, the specific methane production rate of the UABE reactor was 2.08 L/L.d, which was less than that of the theoretically value of a normal upflow anaerobic reactor (Table 6.2). In an ideal anaerobic upflow reactor operating at an OLR of 8.0 g COD/L.d with glucose as a substrate, the methane production rate is theoretically 2.66 L/L.d when the COD removal efficiency is 95% and the methane yield is 0.35 L/g COD_r. In an upflow anaerobic reactor, the influent characteristics directly influence the anaerobic microbial activity at the bottom of the reactor (Pant & Adholeya, 2007). In the UABE reactor, the effluent VFAs was as high as 2,894mg/L for the COD, and the COD removal efficiency was only 82.3%. This indicates that the activity of the methanogenic bacteria at the bottom of the UABE reactor was suppressed by the low pH of the influent. However, the methane production rate of the UASB reactor was 1.21 L/L.d, which was considerably lower than that of the UABE reactor. It is likely that the anaerobic bacteria in the UASB reactor are more sensitive to the acidic influent compared the condition in the UABE reactor.

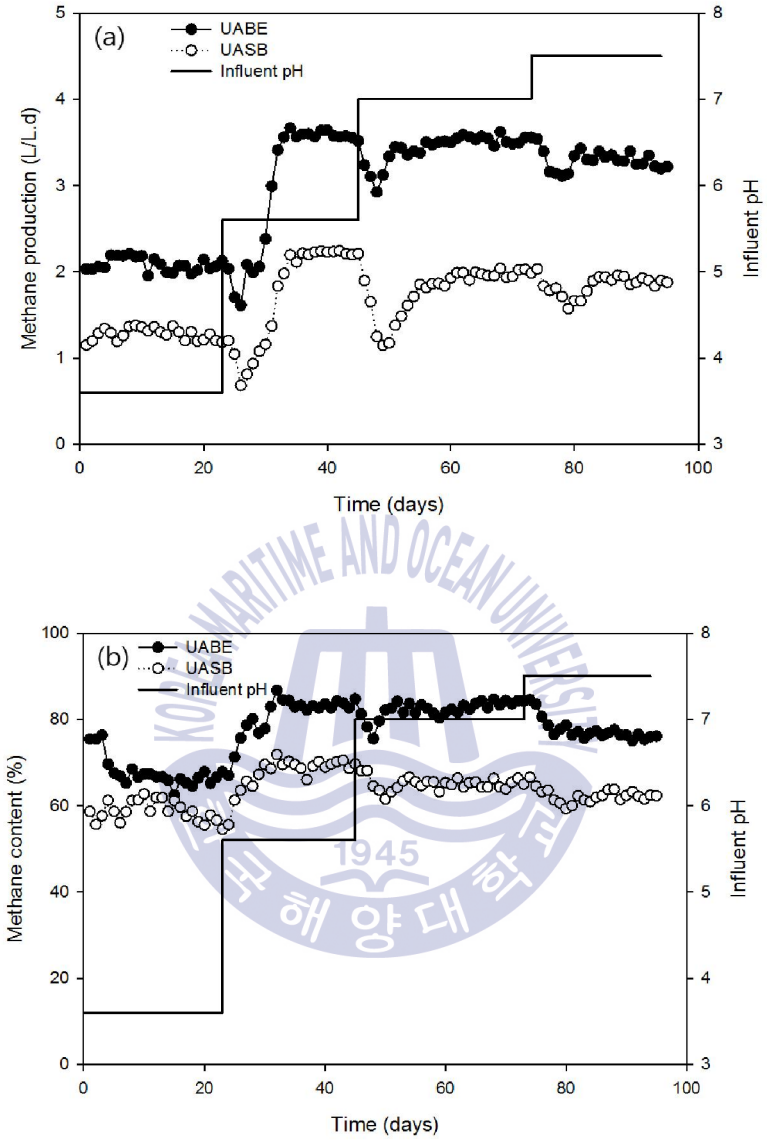


Fig. 6.2. (a) Specific methane production rate and (b) methane content in biogas for the UABE and UASB reactors with distillery wastewater at different influent pHs.

Table 6.2 Performance of the UABE and UASB reactors with distillery wastewater at different influent pHs

Parameter	Reactors	pH 3.6	pH 5.6	pH 7.0	pH 7.5
pH	UABE	7.07±0.02	7.62±0.01	8.01±0.03	8.08±0.01
	UASB	6.95±0.02	7.55±0.03	7.92±0.01	8.04±0.01
Alkalinity (mg/L as $CaCO_3$)	UABE	2,668±70	6,745±46	16,161±135	20,207±70
	UASB	2,524±53	6,230±22	14,899±102	18,690±159
VFAs (mg/L as COD)	UABE	2,894±249	466±57	767±77	903±49
	UASB	3,654±356	784±120	1,335±87	1,450±112
COD removal efficiency (%)	UABE	82.3±0.2	94.9±0.7	96.7±0.1	91.4±0.4
	UASB	77.5±0.3	84.5±0.7	86.7±0.3	83.2±1.2
Sulfate removal efficiency (%)	UABE	42.1±4.0	91.9±0.8	91.1±0.8	85.9±4.0
	UASB	39.0±1.8	59.9±2.3	57.0±5.3	56.4±4.6
Specific methane production rate (mL/L.d)	UABE	2.08±0.05	3.56±0.02	3.53±0.06	3.23±0.09
	UASB	1.21±0.04	2.22±0.02	1.98±0.04	1.90±0.04
eDIET (%)	UABE	2.8	4.6	4.6	4.6
bDIET (%)	UABE	41.4	33.0	39.7	36.6
bDIET methane production (mL/L.d)	UABE	0.86	1.18	1.40	1.18
Methane content (%)	UABE	66.8±1.1	83.6±0.9	84.0±0.6	75.8±0.6
	UASB	56.0±1.23	69.7±0.7	65.0±1.0	62.3±0.6
Methane yield (mL CH_4/gCOD)	UABE	320±7	469±5	463±8	435±15
	UASB	195±1	326±2	289±9	285±4
Energy efficiency (%)	UABE	78.7±1.2	93.1±2.6	90.8±2.2	86.0±1.9
	UASB	46.9±2.9	71.1±1.5	61.7±1.1	61.3±2.7

However, the partial neutralization of the acidic influent to pH 5.6 rapidly increased the methane production rates in both upflow anaerobic reactors, with the production rates reaching 3.56 mL/L.d and 2.22 mL/L.d in the UABE and UASB reactors, respectively. In the UABE reactor, the partial neutralization of the acidic influent increased the effluent pH to 7.62, and the VFAs decreased to 466 mg/L as COD (Table 6.2). The COD removal efficiency levels were also improved significantly to 94.9% and 84.5% in the UABE and UASB reactors, respectively (Fig. 6.3a). During anaerobic digestion, alkalinity is commonly produced by the reduction reactions of methane production and sulfate reduction as well as the decomposition of nitrogenous compounds (Feng et al., 2016a; Song et al., 2016; Song et al., 2004). In the UABE reactor, the partial neutralization of the acidic influent considerably increased the sulfate reduction efficiency as well as the methane production rate (Fig. 6.3b). The sulfate reduction reaction produces sulfide, a substance which inhibits the activity of methanogenic bacteria (Gutierrez et al., 2009; Zhang et al., 2009b). However, an inhibitory effect of sulfide on the production of methane was not observed in the UABE reactor. This likely occurred because the influent sulfate level is approximately 1.5 g/L, which is relatively low compared to the amount of organic matter (Table 6.1). This shows that the alkalinity in the UABE reactor was mainly generated by reduction reactions which occurred when the wastewater passed through the upflow reactor. It is likely that partial neutralization mitigated the inhibition of the acidic low pH for the methanogenic activity, thereby producing more alkalinity and further increases in the pH. It should also be noted that the production of methane was improved more in the UABE reactor, suggesting that a considerable amount of methane in the UABE reactor is produced through the electron transfer pathway, during which the influence of the pH is low. In the UASB reactor, partial neutralization increased the effluent pH to 7.5, which was slightly lower than that in the UABE reactor, and the methane production, alkalinity and COD/sulfate removal efficiency outcomes were much lower than those of the UABE reactor (Table 6.2).

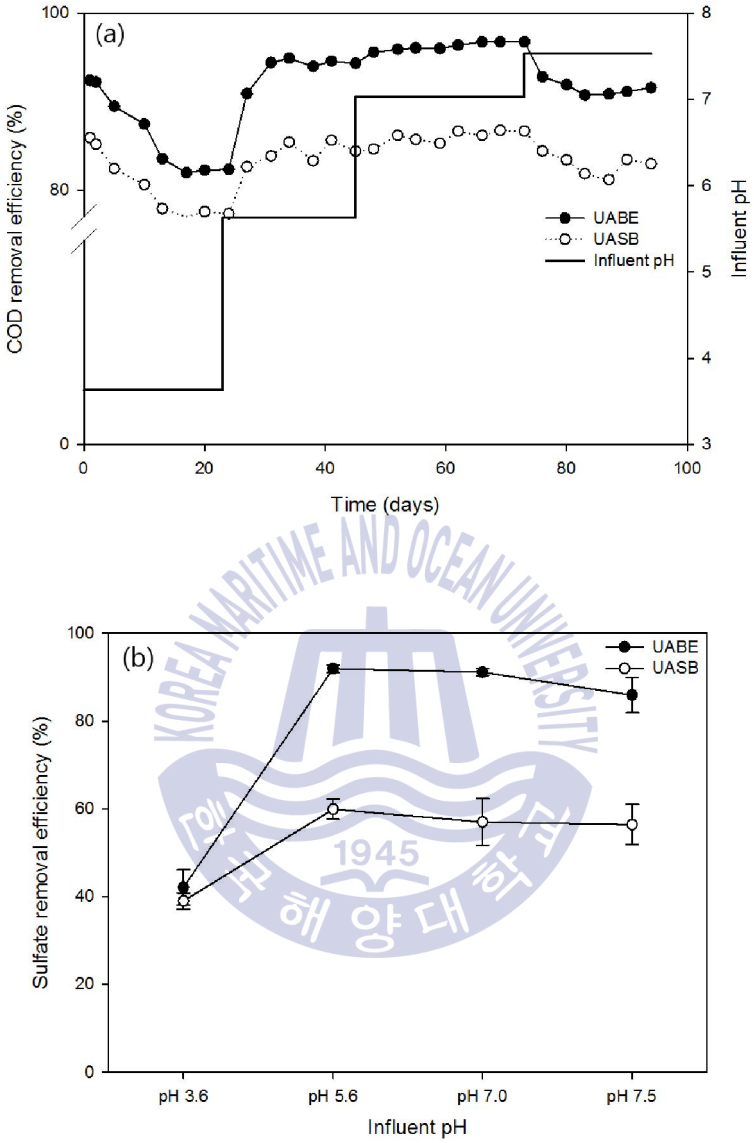


Fig. 6.3. (a) Removal efficiency of COD (a) and sulfate (b) in the UABE and UASB reactors with distillery wastewater at different influent pHs.

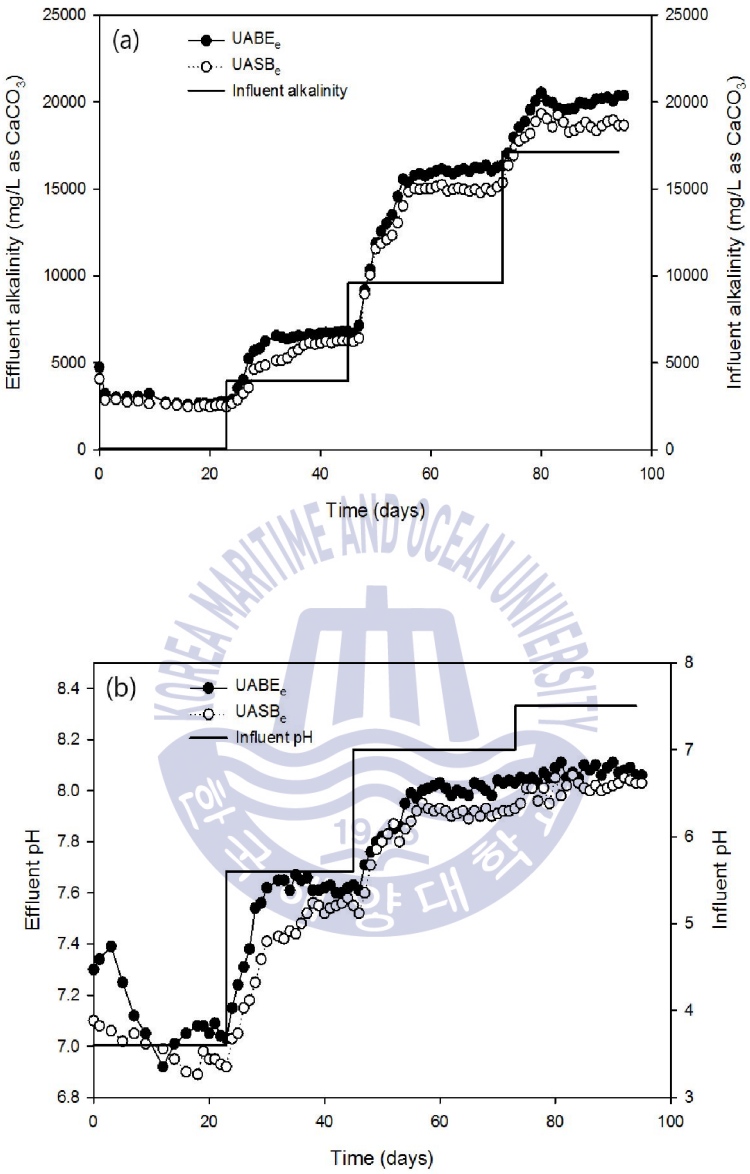


Fig. 6.4. Changes of the effluent (a) alkalinity and (b) pH in the UABE and UASB reactors with distillery wastewater at different influent pHs.

In the UABE reactor, further neutralization of the acidic influent to pH 7.0 and pH 7.5 increased the effluent pH and alkalinity (Fig. 6.4), but the VFA levels of the effluent were also increased and the methane production and energy efficiency outcomes were slightly decreased (Fig. 6.2a). In the UASB reactor, further neutralization also increased the effluent pH and alkalinity (Fig. 6.4), but these values were slightly lower than those in the UABE reactor, and the trend of the neutralization of the acidic influent on the performance was similar to that in the UABE reactor. Considering the alkaline chemical consumption, methane production, and energy efficiency, it is recommended to neutralize the acidic influent to pH 5.6 for an effective treatment of distillery wastewater in anaerobic upflow reactors.

② Electron transfer pathways for methane production

For a better understanding of the influence of the acidic influent in upflow anaerobic reactors, a close examination of the electron transfer pathway for methane production is necessary. The electron transfer pathway for methane production from organic matter during anaerobic digestion can be divided into indirect interspecies electron transfers (IIETs) and direct interspecies electron transfers (DIETs) (Kato, 2015; Zhao et al., 2016; Shen et al., 2016). IIETs are electron transfer pathways that are mediated by anaerobic degradation intermediates such as acetate and hydrogen/formate (iIIET), or by the electron shuttles such as flavin-based compounds, sulfur-based compounds, and humic substances (sIIET) (Shrestha et al., 2014; Dube & Guiot, 2015). DIETs can be classified as biological DIETs between electroactive bacteria and methanogenic bacteria via the outer membrane type C cytochrome /conductive pili (bDIET), electron transfer through the anode and cathode in a bioelectrochemical reactor (eDIET), and electron transfer via a conductive material such as magnetite, activated carbon and/or carbon nanotubes (cDIET) (Kato, 2015; Feng et al., 2016a,b; Zhao et al., 2016). In this study, the distillery wastewater was collected from an ethanol plant that mainly fermented from the barley, tapioca and sweet potato. The conductivity of

distillery wastewater was only 2.59 mS/cm (Table 6.1), which is less than other studies (Samsudeen et al., 2016; Kobya & Delipinar, 2008). It is unlikely that distillery wastewater contained an enough amount of natural conductive materials or electron transfer shuttles that could affect methane production. This indicates that the main electron transport pathways for methane production are the iIIET, bDIET, and eDIET in the UABE reactor (Zhao et al., 2016; Van Eerten-Jansen et al., 2015). For the UASB reactor, it was reported that the main electron transfer pathways is the iIIET via acetate and hydrogen/formate, but a small amount of methane is produced by the bDIET in the anaerobic granular sludge (Kato, 2015; Zhao et al., 2014). However, in the UABE reactor, the methane production via the eDIET was less than 5% of the total methane production (Table 6.2), which was estimated from the electrical current between the anode and cathode. This indicates that the main electron transfer pathways for methane production in the UABE reactor are the iIIET and bDIET, similar to that in the UASB. However, the methane production for UABE reactor was 60-79% higher than the UASB reactor depending on the influent pHs (Fig. 6.2a). It suggests that the bDIET pathway for methane production was significantly improved by the enrichment of electroactive bacteria in the UABE reactor.

Ignoring the small amount of the methane produced through the bDIET pathway in the UASB reactor, the methane production of the DIET pathways in the UABE would be the difference in the methane production between the UABE and UASB reactors. In this case, the methane production via the bDIET in the UABE reactor can be estimated by excluding the methane production of the eDIET pathway from the DIET pathway. Interestingly, in the UABE reactor fed with acidic influent at a pH of 3.6, the methane production rate through bDIET was 0.86 mL/L.d, which was 41.4% of the total methane production (Table 6.2). However, partial neutralization of the acidic influent to pH 5.6 increased the methane production through bDIET to 1.18mL/L.d, which was 33.0% of the total methane production in the UABE reactor. This indicates that IIET for methane production, which was greatly

inhibited by a low pH, was improved with an increase in the influent pH, also therefore indicating that the electroactive bacteria for bDIET is less susceptible to a low pH than the anaerobic bacteria for IIET. This is consistent with the findings of previous studies, in which the performance of bioelectrochemical anaerobic digestion was less sensitive to a low pH and temperature than that of conventional anaerobic digestion (Feng et al., 2016b; Yuan et al., 2011).

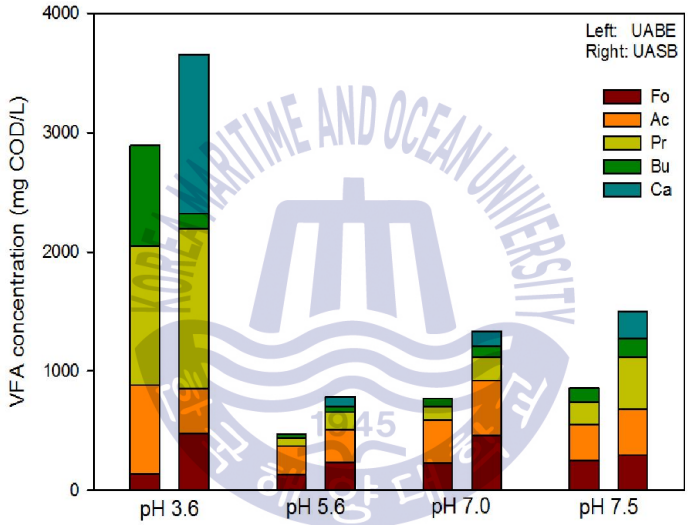


Fig. 6.5. (a) Effluent VFAs in the UABE and UASB reactors with distillery wastewater at different influent pHs.

In the UABE reactor, the main components of VFAs were the short-chain fatty acids (SCFAs) such as formate, acetate, propionate and butyrate, but long-chain fatty acids (LCFAs) such as caproate were observed in the effluent of the UASB reactors (Fig. 6.5). Generally, LCFAs are difficult to convert to methane, and they also inhibit methane production during anaerobic digestion (Hanai et al., 1981; Koster et al. 1987). The formate concentration in the UABE reactor was always lower than that in the UASB reactor. This

indicates that the accumulated amount of hydrogen/formate that is generated at a higher $NADH/NAD^+$ ratio is lower in the UABE reactor. A form of IIET for methane production is the electron transfer pathway that uses the electrons from hydrogen/formate to reduce carbon dioxide (Mir et al., 2016; Shrestha et al., 2014). This indicates that methane production from the IIET pathway is relatively reduced by the increase of the DIET pathway via electroactive bacteria in the UABE reactor.

③ Methane content in biogas and the methane yield

The amount of methane produced as the final product during conventional anaerobic digestion is determined by the characteristics of the substrate, as established by the Buswell equation (Song et al., 2016; Shen et al., 2016). For example, when the substrate is glucose, methane and carbon dioxide are theoretically produced at same amount and rate during the anaerobic digestion process. However, the IIET, which consists of several stages of enzymatic reactions with losses, has low electron transfer efficiency (Kato, 2015; Dube & Guiot, 2015). Therefore, methane production through IIET in an actual case of anaerobic digestion is lower than that of carbon dioxide. On the other hand, the solubility of carbon dioxide in water is considerably higher than that of methane and is higher at a higher pH in a bulk solution. Thus, the methane content in biogas during conventional anaerobic digestion is approximately 50-65%, which is higher than the carbon dioxide content (Song et al., 2004; Kardos et al., 2011). Recently, bDIET was found to have higher electron transfer efficiency levels than IIET (Kato, 2015; Zhao et al., 2016), indicating that methane production through bDIET is higher than that through IIET. In previous studies, the methane content in biogas during bioelectrochemical anaerobic digestion was found to be in the range of 73-81%, significantly higher than that during conventional anaerobic digestion (Song et al., 2016). The methane content in biogas from the UABE reactor fed with acidic influent at pH 3.6 was 66.8%, which was approximately 10% higher than that of the UASB reactor (Fig. 6.2b). However, the methane

content in biogas was increased to 84.0% in the UABE reactor after the neutralization of the acidic influent to pH 7.0, which is higher than the value of 65.0% for the UASB reactor (Table 6.2). Interestingly, methane production through eDIET, which was produced from the electrodes in the UABE reactor, was less than 5% of the total methane production (Table 6.2). This suggests that more methane in the UABE than in the UASB was produced through the improved bDIET in the UABE reactor.

The methane yield, referring to methane production from 1g of COD_r during anaerobic digestion, is an indicator of the electron transfer efficiency from the substrate to methane. The methane yield was 320 mL/g COD_r when acid influent at pH 3.6 was fed into the UABE reactor, but it increased to 469 CH_4 mL/g COD_r when the influent wastewater was neutralized to pH 5.6 (Table 6.2). It is well known that the theoretical methane yield in the anaerobic digestion is 350 mL CH_4/g COD_r from the Buswell equation. However, some methane yields of the UABE reactor were higher than the theoretical value. The methane yield could be partially increased by the activation of bDIET with high electron transfer efficiency. However, it seems that the influent distillery wastewater contains some reduced substances that are not detected by the COD analysis, and the additional methane was produced by the reduction of carbon dioxide by donating the electrons from these reduced substances. Ammonia is one of the candidate reduced substances that used as an electron donor for additional methane production in the UABE. It is reported that ammonia can be an electron donor for hydrogen production in bioelectrochemical system (Zhan et al., 2014). However, the neutralization of the acidic influent to pH 7.5 increased the effluent pH to 8.08 in the UABE reactor (Fig. 6.4b), and the performance of the UABE reactor as estimated by the COD removal efficiency rate; the total methane production; and the bDIET methane production, methane yield and methane content in methane productions biogas showed a slight decrease (Table 6.2). This indicates that the methanogenic activity in the UABE reactor was slightly inhibited by the pH higher than 8.0. For the UASB

reactor, the methane yield was varied in range of 0.195-0.326 L CH_4/g COD_r depending on the influent pHs, which was always lower than that of the UABE reactor. The energy efficiency, referring to the percentage of energy recovered as methane relative to the sum of the energy contained in the COD removed and the electrical energy consumed, is proportional to the methane yield. For the acidic influent at pH 3.6, the energy efficiency levels were 78.7% and 46.9% for the UABE and UASB reactors, respectively (Table 6.2). This indicates that methane in the UABE reactor, in which the electroactive bacteria were more enriched, was produced more through the DIET pathway, which was less affected by the acidic pH. The energy efficiency in the UABE reactor was increased to 93.1% by the partial neutralization of the acidic influent to pH 5.6. This outcome indicates that the partial neutralization of the acidic influent to pH 5.6 increases the utilization of the substrate and decreases the energy loss by increasing the methane production through the DIET pathways (Kato, 2015; Zhao et al., 2016). However, the neutralization of the acidic influent to pH 7.0 and pH 7.5 reduced the energy efficiency levels to 90.8% and 86.0%, respectively, due to the decrease in the methane yield (Table 6.2).

④ eDIET and bDIET for methane production

The EIS data in the Nyquist representation were well fitted into the Randle equivalent circuit model (Fig. 6.6). This suggests that the methane production in the UABE reactor was mainly controlled partly by the charge transfer and partly controlled by diffusion on the anode surface. The estimated values of the solution ohmic resistance (Rs), charge-transfer resistance (Rct), Warburg impedance (W), and capacitance (C) are presented in Table 6.3. The ohmic resistance of the solution for the anode was in the range of 1.18-1.48 Ω, similar to that of the cathode (Table 6.3). This finding indicates that the electric properties in the solution were not significantly changed by the influent pH condition. The charge transfer resistance for the cathode ranged from 1.63 to 1.82 Ω, also not greatly affected by the neutralization of the

acidic influent. However, the charge transfer resistance of the anode was considerably affected by the influent pH of the distillery wastewater. For the acidic influent at a pH of 3.6, the charge transfer resistance for the anode was significantly high, at 31.42 Ω, after which it was drastically reduced to the range of 5.67-7.89 Ω by the neutralization of the acidic influent (Table 6.3). Thus, with the acidic influent of pH 3.6, methane production via eDIET is determined by the activity of the electroactive bacteria on the anode. This is in agreement with the methane production via eDIET from the neutralized acidic influent of the distillery wastewater. For the acidic influent at a pH of 3.6, methane production from the electrode was only 2.8% of the total methane production, and the neutralization of the acidic influent until the pH exceeded 5.6 increased the rate to 4.6% (Table 6.2). Methane production via the eDIET pathway was associated with the electron transfer activity of the electroactive bacteria attached onto the surface of anode (Wang et al., 2009; Feng et al., 2016b). This indicates that the activity of electroactive bacteria can also be affected by the low pH of the acidic distillery wastewater. However, for the acidic distillery wastewater at a pH of 3.6, methane production rates through bDIET and eDIET were 41.4% and 2.8% of the total methane production, respectively (Table 6.2). This suggests that the methanogenic bacteria associated with IIET is more sensitive to the pH than the electroactive bacteria for methane production through DIET.

Table 6.3 EIS data for anode and cathode in the UABE reactor with distillery wastewater at different influent pHs

Contents	Anode				Cathode			
	pH 3.6	pH 5.6	pH 7.0	pH 7.5	pH 3.6	pH 5.6	pH 7.0	pH 7.5
Rs (Ω)	1.48	1.44	1.33	1.18	1.41	1.3	1.18	1.16
Rct (Ω)	31.42	5.67	6.22	7.89	1.63	1.70	1.68	1.82
C (mF)	0.33	0.21	0.32	0.30	3.92	7.67	1.64	1.66
W($1/\Omega\sqrt{Hz}$)	1.57	1.02	3.69	1.19	3.88	6.32	8.96	7.53

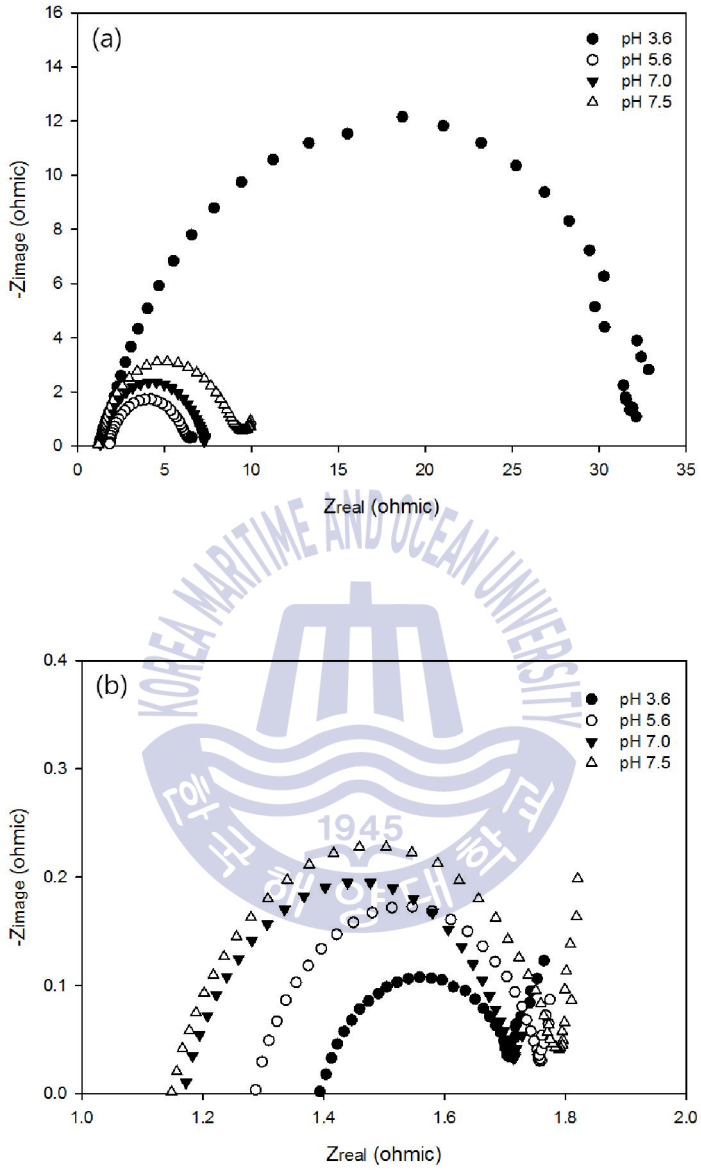


Fig. 6.6. Nyquist plot of EIS data for (a) the anode, and (b) the cathode in the UABE reactor with distillery wastewater at different influent pHs.

6.3.2 Influence of effluent recirculation on upflow anaerobic bioelectrochemical reactor

① Methane productions from UABE and UASB reactors

The UASB reactor is one of the popular high-rate anaerobic digestion processes for treating high-strength organic wastewater. The methane production rate of the UASB reactor varies, depending on the type and nature of the substrate (Habeeb et al., 2011; Kaviyarasan, 2014). In the UASB reactor treating acidic distillery wastewater of pH 3.6, the methane production rate was 1.21 L/L.d at a loading rate of 8 g COD/L.d (Fig. 6.7a). However, the methane production rate from the UABE reactor was 2.08 L/L.d, which was 71.9% higher than the UASB. The optimum pH for the growth of methanogenic bacteria in anaerobic digestion is in the range of 6.6~7.6 (Reungsang et al., 2016; Ritmann & McCarty, 2001), and the optimum pH range for the acidogenic fermentation bacteria is from 4.0 to 6.0 (Bengtsson et al., 2008; Zheng et al., 2015). This means that the methanogenic bacteria are more sensitive to low pH than the acidogenic bacteria. The pH of the UABE reactor effluent was 7.07, which was slightly higher than the 6.95 of the UASB (Fig. 6.8a). This indicates that the methanogenic reaction in the UASB was more affected by low pH than the UABE. The hydraulic characteristics of the flow in UASB could be described as a dispersed plug flow, in which the physicochemical properties of the influent wastewater changes continuously, while moving from bottom to top (Kalyuzhnyi et al., 2006; Kaviyarasan, 2014; Ritmann & McCarty, 2001). This means that due to the inflow of the acidic wastewater, the anaerobic microorganisms in the lower part of the reactor are directly exposed to the lower pH value, but the acidity was gradually neutralized by the alkalinity generated while moving to the upper part of the reactor. The alkalinity of the UASB reactor effluent was about 2,520 mg/L as $CaCO_3$, but that of the UABE reactor effluent was slightly higher at 2,670 mg/L as $CaCO_3$ (Fig.

6.8b). The alkalinity of the anaerobic digester is mainly generated by the ammonia from the decomposition of nitrogen compounds, the reduction of organic acids and carbon dioxide to form methane, and the sulfate reduction reaction (Feng et al., 2016a; Song et al., 2016; Song et al., 2004). The concentration of VFA in the UASB effluent was about 3,650 mg/L as COD. This is the result of the decrease in the methanogenic activity by exposure to low pH while the acidic wastewater passes through the granule layer in the UASB reactor. The VFA in the UABE effluent was 2,890 mg/L as COD, which was lower than the UASB effluent (Fig. 6.9). The higher values in pH and alkalinity, and the lower VFA in the UABE effluent indicate that the methanogenic bacteria in the UABE reactor was less inhibited by the low pH than the UASB reactor. It is well known that electroactive bacteria are enriched in the anaerobic reactor coupled with bioelectrochemical technology (Lovley, 2011; Shen et al., 2016; Feng & Song, 2016a). The DIET pathways were possibly activated for methane production in the UABE reactor by the electroactive bacteria. It suggests that the DIET for methane production is less sensitive to the low pH than the IIET.

On the other hand, the sulfate reduction rate in the UASB was 39%, which was slightly less than the 42.1% of the UABE (Table 6.4). Although the hydrogen sulfide content in biogas was not monitored, the sulfate content was only 1.6 g/L in the influent wastewater. The sulfide levels in the effluent were from 0.036 g/L to 0.041 g/L which were estimated from the sulfate reductions for both upflow reactors at RR0. The inhibitory level of sulfide is in the range of 0.1~0.8 g/L in the anaerobic digester (Parkin et al., 1990). It seems that the inhibitory effect of sulfide was not serious in the both upflow reactors. However, for the anaerobic treatment of the distillery wastewater with high sulfate content, the sulfide dissolved in the bulk liquid could be a serious substance inhibiting the methanogenic activity (Mohana et al., 2009; Krishnamoorthy et al., 2017).

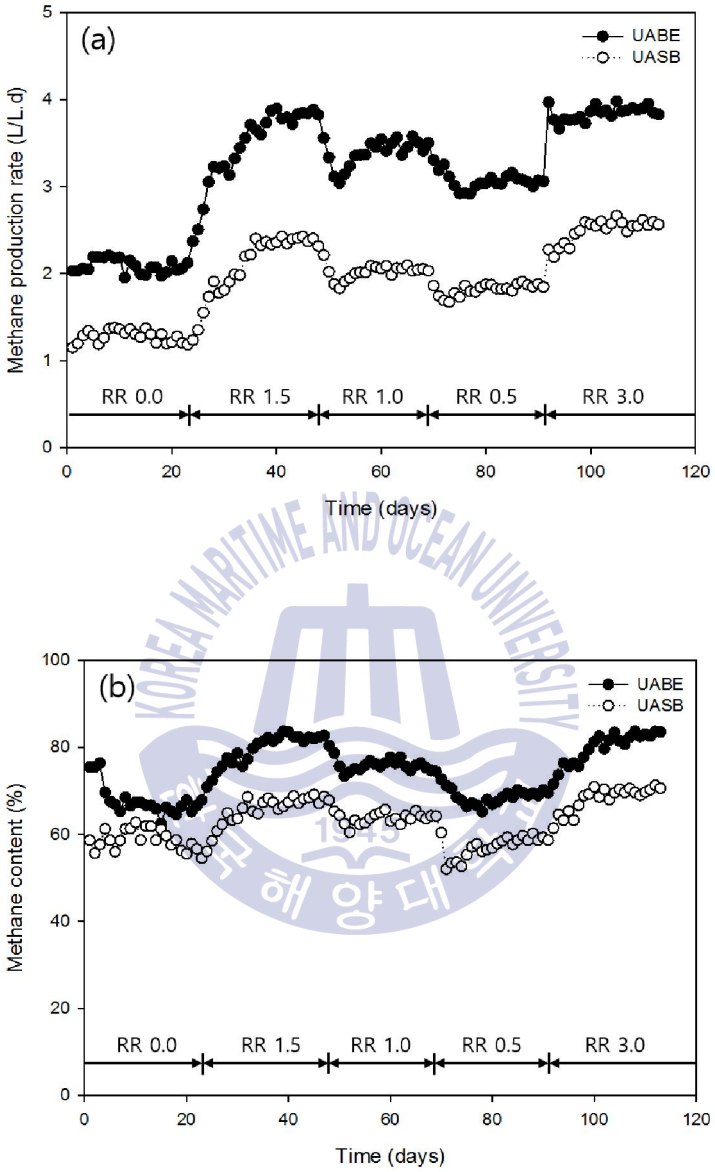


Fig. 6.7. (a) Methane production rate, and (b) methane content, in biogas at different recirculation ratios of the UABE and UASB reactors.

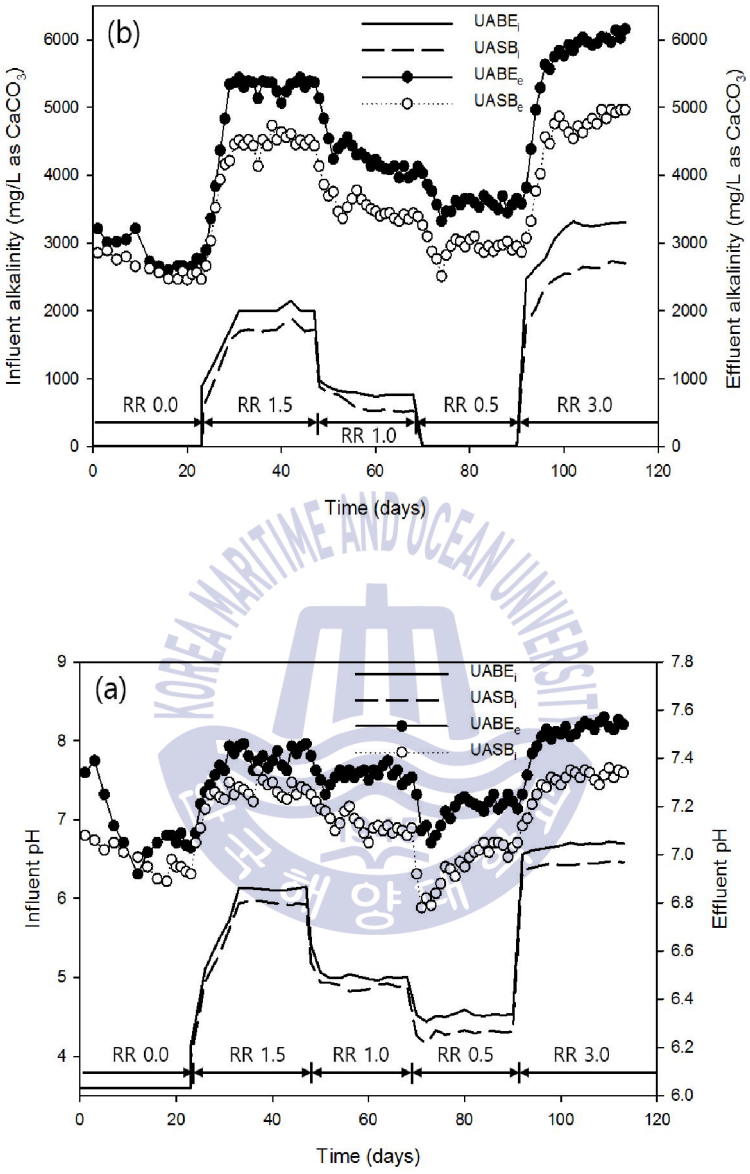


Fig. 6.8. Behaviors of (a) pH, and (b) alkalinity, with changes of the recirculation ratios of the influent and effluents in the upflow reactors.

Table 6.4 Performance of the UABE and UASB reactors at different recirculation ratios

	Reactors	RR 0.0	RR 0.5	RR 1.0	RR 1.5	RR 3.0
pH	UABE	7.07±0.02	7.22±0.03	7.32±0.03	7.42±0.05	7.55±0.02
	UASB	6.95±0.02	7.03±0.03	7.10±0.01	7.27±0.03	7.34±0.02
Alkalinity (mg/L as $CaCO_3$)	UABE	2,668±70	3,590±84	4,041±78	5,366±57	6,035±72
	UASB	2,524±53	2,962±53	3,381±55	4,511±37	4,922±61
VFAs (mg/L as COD)	UABE	2,894±249	711±57	506±47	334±57	318±54
	UASB	3,654±356	1002±18	839±20	885±61	746±45
COD removal rate (%)	UABE	82.3±0.2	87.4±0.4	92.8±0.4	96.5±0.1	96.3±0.6
	UASB	77.5±0.3	79.4±0.4	84.5±0.8	87.7±0.4	90.8±0.4
Sulfate removal rate (%)	UABE	42.1±4.0	77.2±1.1	83.3±2.2	91.3±1.6	93.2±0.6
	UASB	39.0±1.8	45.3±2.6	52.8±1.4	64.1±5.2	70.8±3.8
Specific methane production rate (L/L.d)	UABE	2.08±0.05	3.05±0.03	3.49±0.06	3.82±0.06	3.88±0.05
	UASB	1.21±0.04	1.87±0.03	2.05±0.03	2.40±0.02	2.54±0.04
Percentage of methane produced from cathode (eDIET, %)	UABE	2.80±0.04	3.80±0.03	3.80±0.04	3.60±0.01	3.70±0.08
Methane content (%)	UABE	66.8±1.1	69.4±0.5	75.3±0.8	82.1±0.5	83.0±0.5
	UASB	56.0±1.23	59.1±0.7	64.3±0.7	68.3±0.8	70.1±0.9
Methane yield (mL CH_4/gCOD)	UABE	320±7	439±1	462±5	500±4	501±6
	UASB	195±1	295±5	307±1	342±2	345±4
Energy efficiency (%)	UABE	78.0±1.2	83.6±2.2	90.9±1.2	97.7±1.5	96.6±1.2
	UASB	46.9±2.9	62.2±2.5	65.2±2.2	74.3±3.3	75.9±1.6

RR: Recirculation Ratio

② Intermediates and the electron transfer pathway

In anaerobic digestion, the efficiency of electron transfer from substrate to methane differs greatly, depending on the pathway of electron transfer. Therefore, the concentration of the intermediate products and the ratio of the constituents that depend on the $NADH/NAD^+$ ratio give insight into understanding the electron transfer pathways (Feng et al., 2016a; Lyberatos & Skiadas, 1999; Ren et al., 2002). In this study, the VFA of the UASB effluent was as high as 3,650 mg/L as COD, and the major components were formate of 480 mg/L as COD, propionate of 1,335 mg/L as COD, and caproate of 1,336 mg/L as COD (Fig. 6.9). In conventional anaerobic digestion, the electron transfer pathways from substrate to methane have been described by the IIET using intermediates, such as acetate, hydrogen and formate. However, it was recently reported that electroactive bacteria, such as *Geobacter metallireducens* and *Geobacter Sulfurreducens*, can transfer electrons directly to acetoclastic methanogens, such as Methanosaeta and Metanosarcina, through outer membrane c-type cytochromes and conductive pilus-like structures in the bioelectrochemical reactor, which is referred to as biological direct interspecies electron transfer (bDIET) (Kouzuma et al., 2015; Shen et al., 2016; Zhao et al., 2016). The bDIET for methane production has also been observed to occur in the granules of the UASB reactor (Kouzuma et al., 2015; Shen et al., 2016; Zhao et al., 2016), and is commonly more advantageous than the IIET in terms of energy transfer efficiency (Dube & Guiot, 2015; Shen et al., 2016). However, the electron transfer pathways for methane production can vary, depending on environmental and operational conditions, such as pH, temperature, substrate type and organic and hydraulic loading rate. In the UASB effluent, high concentrations of formate, propionate, and caproate indicate that the IIET pathways for methane production were probably more inhibited by the low pH of the incoming acidic distillery wastewater.

Fig. 6.9 shows that the accumulated VFA in the UABE effluent was 2,894 mg/L as COD, which was lower than the 3,654 mg/L as COD of the UASB.

The major components of VFA in the UABE effluent were acetate, propionate and butyrate, but caproate was not detected. However, the formate was only 138 mg/L as COD, which was much lower than that of the UASB reactor 480 mg/L as COD. This means that the accumulation of hydrogen/formate, that is, the NADH/NAD^+ ratio, in the UABE reactor was lower than that in the UASB reactor. The electroactive bacteria could be easily enriched in a bioelectrochemical reactor equipped with anode and cathode (Zhao et al., 2016). In previous studies, the increase in methane production from anaerobic digestion coupled with bioelectrochemical device was commonly described by the bioelectrochemical reduction of carbon dioxide to methane on the cathode surface (Villano et al., 2016; Wang et al., 2009; Zhao et al., 2014). The methane production from the carbon dioxide reduction on the surface of cathode is proportional to the Faraday current flowing between cathode and anode. However, the methane production from the bioelectrochemical reduction of carbon dioxide on the cathode that was estimated from the current is only about 2.8% of the total methane production (Table 6.4). It is possible that there are other important electron transfer routes for methane production in the UABE, rather than direct interspecies electron transfer through the electrode (eDIET) (Feng et al., 2016a; Song et al., 2016). In anaerobic digestion, electroactive bacteria can transfer electrons directly to the methanogenic bacteria through conductive materials, such as activated carbon, graphite particles and magnetite, which is referred to as cDIET (Kouzuma et al., 2015; Shen et al., 2016). However in this study, it is unlikely that the conductive materials were significantly present in the acidic wastewater. The UABE reactor demonstrates that the electroactive bacteria were more enriched in the bulk of digestion solution than the surface of the electrode, and the enriched electroactive bacteria in the digestion solution considerably improved the electron transfer for methane production.

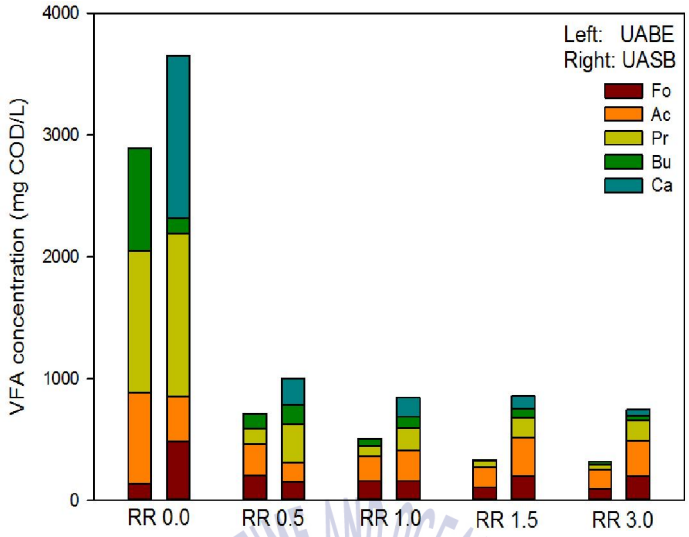


Fig. 6.9. Levels of VFA components at different recirculation ratios of the UABE and UASB reactor effluents.

③ Methane content in biogas

The methane content of the biogas in the UABE reactor was 66.8%, which was significantly higher than the 56% of the UASB reactor (Fig. 6.7b). This result is in good agreement with several previous studies that the methane content in biogas in bioelectrochemical anaerobic reactors is higher than in conventional anaerobic reactors (Feng et al., 2016a; Song et al., 2016). The theoretical value of the methane yield in anaerobic degradation of organic matter is determined by the substrate composition, as can be calculated by the Buswell equation (Shen et al., 2016; Song et al., 2016). However, the solubility of carbon dioxide as a major component of biogas is higher than methane, and it increases with pH of the solution. The pH in anaerobic digesters can be increased by the increase in alkalinity (Song et al., 2004). Therefore, the amounts of methane, sulfide and alkalinity, which are the reduction products of the carbon dioxide or sulfate, increase with the increase in the electron transfer efficiency for methane production, and the pH

increases as well. This means that the increase of the electron transfer efficiency of methane production from substrate in the anaerobic digestion can increase the methane content in the biogas . However, the loss of electron transfer carriers inevitably occurs in the electron transfer reaction that takes place in the anaerobic decomposition of organic matter (Kato, 2015; Shen et al., 2016; Zhao et al., 2016). Due to these electron transfer losses, the methane yield in the anaerobic digestion is generally lower than the theoretical value. The electron transfer efficiency for methane production through the DIET is commonly higher than the IIET (Kato, 2015; Shen et al., 2016; Zhao et al., 2016). Methane yield in anaerobic digestion is a good indictor showing electron transfer efficiency from substrate to methane. The methane yield of the UABE reactor was about 320 mL/g COD_r, which was significantly higher than the 195 mL/g COD_r of UASB (Table 6.4). This indicates that the DIET with higher electron transfer efficiency was the dominant electron transfer pathway for methane production in the UABE.

④ Dependence of the DIET on the effluent recirculation

The methane production of the UABE reactor was significantly improved by the effluent recirculation (Fig. 6.7a). When the effluent recirculation ratio was 0.5, the methane production in the UABE reactor was 3.05 L/L.d, which was significantly higher than the 2.08 L/L.d in the effluent non-recycled reactor (Table 6.4). As the effluent recirculation ratio increased to 3.0, the methane production rate further increased to 3.88 L/L.d. At 0.5 of the effluent recirculation ratio, the effluent alkalinity of the UABE reactor was 3,590 mg/L as $CaCO_3$, and when the recirculation ratio was increased to 3.0, it increased to 6,040 mg/L as $CaCO_3$ (Table 6.4). Various reduction reactions in the anaerobic digestion are involved in alkalinity production (Song et al., 2016; Song et al., 2004). However, the alkalinity increase with the effluent recirculation ratio was linearly proportional to the methane production rate (Fig. 6.10a). This implies that the alkalinity in the UABE was mainly increased by the carbon dioxide reduction to methane. When the recirculation

ratio increased from 0.5 to 3.0, the pH of the UABE reactor effluent increased from 7.22 to 7.55, and the VFA decreased from 711 ± 57 to 318 ± 54 mg COD/L (Table 6.4). It is possibly that the effluent recirculation in the UABE reactor increases the upflow velocity, and gradually changes the hydraulic flow characteristics from PFR to CSTR. Thus, the effluent recirculation neutralizes the acidity of the incoming acidic wastewater, and relieves the accumulation of intermediate products in the reactor, such as organic acids. This indicates that the effluent recirculation alleviated the inhibitory effect of low pH and high VFA. In the UASB, the methane production rate also increased in proportion to the recycle ratio, but in the recirculation ratio of 3.0, was 2.54 L/L.d, which was only 65.5% of that in the UABE reactor. When the recirculation ratio of the UASB effluent was increased from 0.5 to 3.0, the pH increased from 7.03 to 7.34, and the VFA level decreased from 3,653 to 750 mg COD/L (Table 6.4). The concentrations and compositions of VFAs varied with the effluent recirculation ratio (Fig. 6.9). This indicates that the effluent recirculation ratio affected the electron transfer pathway from the substrate to methane. It is possible that the IIET using acetate, hydrogen and formate, and the bDIET are the main electron transfer pathways for methane production in the UABE reactors, as well as in the UASB reactors, for acidic wastewater treatment. However, caproate was not observed in the UABE reactor effluent. This indicates that the increase in the effluent circulation further enriched the electroactive bacteria, and improved the bDIET. In addition, as the effluent recirculation increased, the concentrations of formate, acetate, propionate and butyrate in the VFA gradually decreased (Fig. 6.9). This suggests that the methane production through improved bDIET alleviated the burden of the IIET for methane production. The effluent recirculation of 0.5 in the UASB reactor significantly reduced the VFA components, including formate, acetate, propionate, butyrate and caproate. However, in the higher effluent recirculation ratio, there was no remarkable reduction in formate and acetate. This implies that the IIET pathway, which produces methane using the intermediates, such as acetate and hydrogen/formate, was the rate-limiting step of the electron transfer for

methane production in the UASB reactor. However, when the recirculation ratio increased from 0.5 to 3.0, the propionate, butyrate and caproate decreased slightly. This means that as the effluent recirculation increased, the VFA fermentation was improved in the UASB reactor, indicating that some methane was produced through the DIET pathway.

The methane yield in the anaerobic reactor is directly related to the efficiency of electrons transferred from the substrate to methane. The result that the higher the electron transfer efficiency, the greater the methane content of the biogas, is very interesting. This effect is due to the difference in the amount and solubility of methane and carbon dioxide produced by anaerobic digestion of organic matter. At the effluent recirculation ratio of 0.5, the methane yield and biogas methane content in the UABE reactor were 439 mL/g COD_r and 69.4%, respectively, which were significantly higher than the 295 mL/g COD_r and 59.1% of the UASB reactor (Table 6.4). The methane yield in the UABE reactor increased with the effluent circulation ratio, and at the recirculation ratio of 3.0, the methane yield and biogas methane content were as high as 501 mL/g COD_r and 83.0%, respectively. It seems that the electron transfer efficiency of the DIET pathway in anaerobic digestion is higher than that of the IIET via acetate, hydrogen/formate (Kouzuma et al., 2015; Rotaru et al., 2014b; Shen et al., 2016). Thus, the greater the amount of electron transfers through the DIET pathway, the lower the proportion of electron transfer through the IIET (Rotaru et al., 2014b). This indicates that the effluent recirculation in the UABE reactor has shifted the electron transfer pathway from the IIET into the more efficient DIET. This suggests that the neutralization of the acidity of the influent wastewater and the increase of the upflow velocity of the wastewater through the effluent recycle in the UABE reactor preferentially promote the enrichment of electroactive bacteria. In the UASB reactor, the methane yield and methane content also increased with the effluent recirculation ratio, but were significantly lower than those of the UABE reactor. This demonstrates that the enriched electroactive bacteria shifted the main electron transfer pathway

in the UABE reactor from IIET to DIET, and the methane production rate in the UABE reactor was higher than that in the UASB reactor.

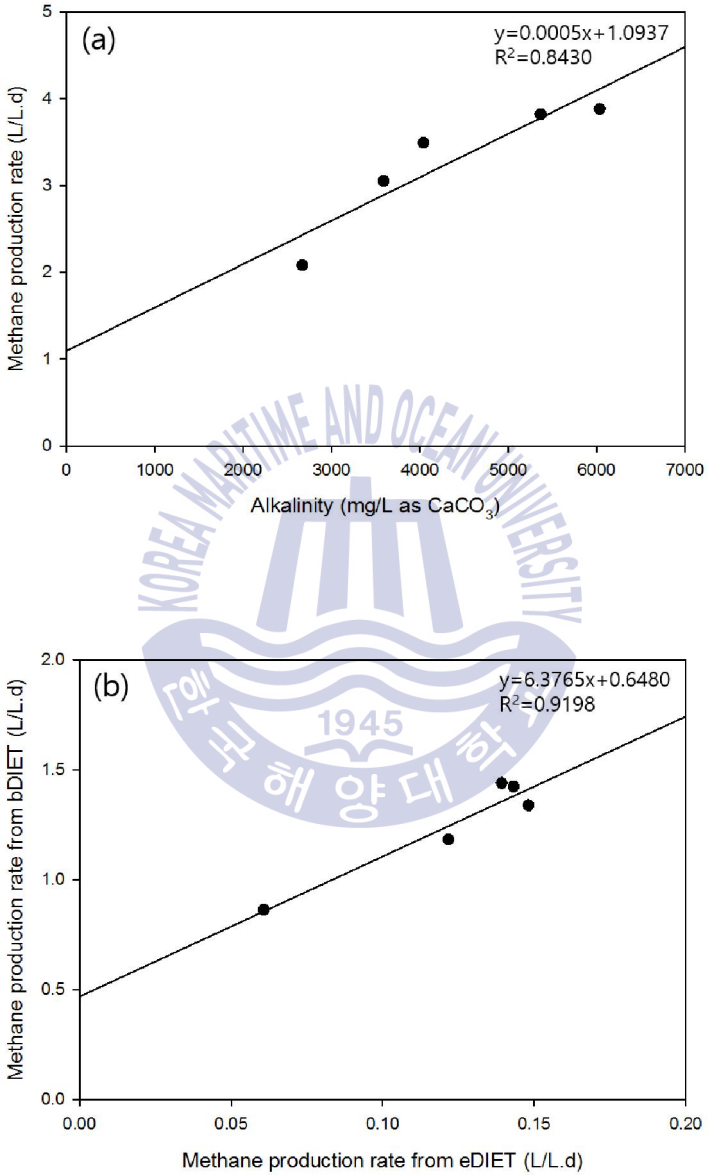


Fig. 6.10. (a) Dependence of methane production rate on the alkalinity, and (b) relationship of the methane produced from bDIET and eDIET in the UABE reactor.

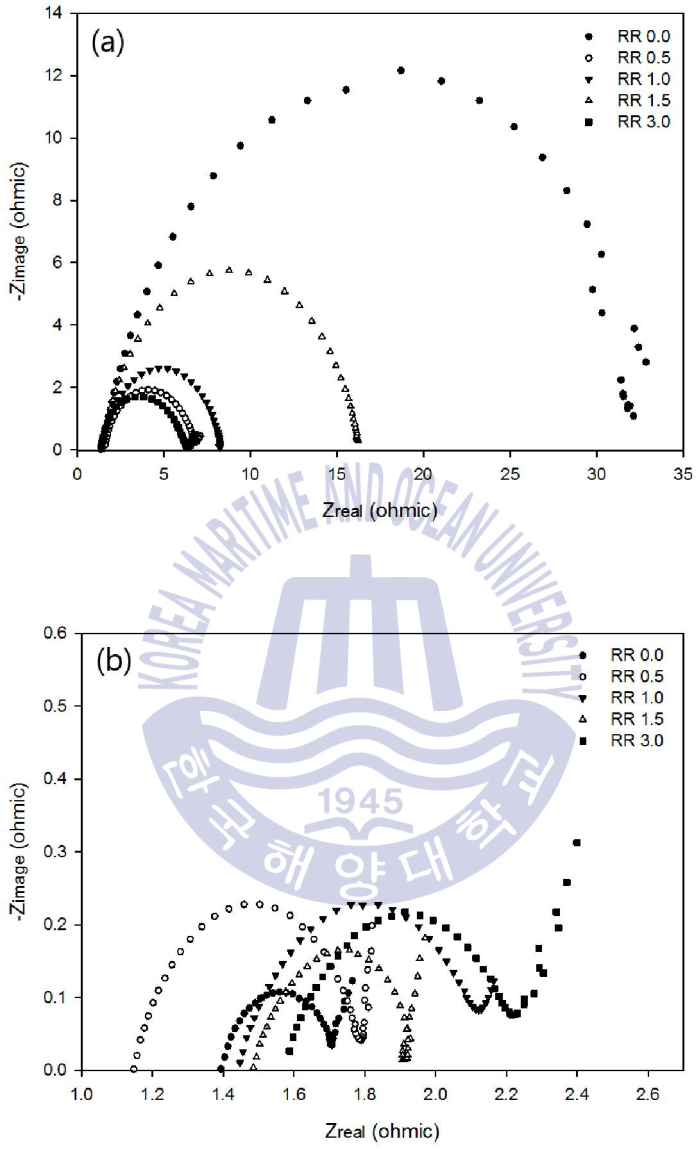


Fig. 6.11. Nyquist plots of EIS data for the (a) anode, and (b) cathode, at different recirculation ratios of the UABE reactor effluent.

Fig. 6.10b shows the relationship between the methane productions in a UABE reactor through the total DIET and eDIET pathway. The total methane production through the DIET in the UABE reactor is the difference between the total methane productions in the UABE and UASB reactors. The amount of methane produced from the eDIET was theoretically estimated by the electric current in the external circuit between the anode and cathode in the UABE reactor. At a recirculation ratio of less than 1.5, total methane production via the DIET was proportional to the methane production by eDIET. It is interesting that the electron transfer through the electrode is associated with the enrichment of electroactive bacteria. This is in agreement with previous studies that electrodes with voltage supply installed into the anaerobic digester facilitated the enrichment of electroactive bacteria (van Eerten-Jansen et al., 2015; Zhao et al., 2016). However, at the recirculation ratio of 3.0, the methane production through the DIET in the UABE reactor no longer increased.

A Randle equivalent circuit model well fitted the EIS data for the anode and the cathode in the UABE reactor in Nyquist plot (Fig. 6.11). This suggests that the methane production in the UABE reactor was mainly controlled by charge transfer, and partly by diffusion on the electrode surface (Feng & Song, 2016a,b). When the acidic distillery wastewater was fed into the UABE reactor without the effluent recirculation, the charge transfer resistance of the anode was 31.4 Ω, which was about 20 times higher than the 1.63 Ω of the cathode (Table 6.5). This indicates that the electron transfer reaction on the anode governed the methane production through the eDIET. It is also shown that electroactive bacteria involved in the DIET reaction of UABE reactors that treat acidic wastewater are a little more sensitive to low pH values than the methanogenic bacteria at the cathode. Increasing the recirculation ratio from 0.5 to 3.0 decreased the charge transfer resistance of the anode from about 14.8 to 5.7 Ω (Table 6.5). However, the charge transfer resistance of the cathode was not significantly different in the range of 1.89 to 2.37 Ω. This indicates that the recirculation of the effluent

improved the activity of electroactive bacteria inhibited at low pH, and improved the methane production through the DIET. At the effluent recirculation ratio of 3.0, the Warburg resistance of the anode and the cathode increased to 13.14 and 21.36, respectively (Table 6.5). The Warburg resistance is related to the concentration polarization, and describes the phenomenon that the reaction at the electrode surface is limited by the deficiency of available substrate (Feng & Song, 2016a). This shows that at the effluent recirculation ratio of 3.0, the methane production in the UABE was not continuously increased, due to the available substrate deficiency.

Table 6.5 EIS data of electrodes in the UABE reactor at different recirculation ratio

Contents		Solution ohmic resistance (Ω)	Charge-transfer resistance (Ω)	Capacitance (uF)	Warburg resistance ($1/\Omega\sqrt{Hz}$)
Anode	RR0.0	1.48	31.4	328	1.573
	RR0.5	1.39	14.84	306	0.286
	RR1.0	1.31	7.01	440	0.225
	RR1.5	1.36	5.88	258	0.985
	RR3.0	1.37	5.73	206	13.135
Cathode	RR0.0	1.41	1.63	3,920	3.88
	RR0.5	1.51	1.89	1,568	10.08
	RR1.0	1.42	2.08	1,998	3.064
	RR1.5	1.31	1.65	2,631	9.362
	RR3.0	1.40	2.37	2,359	21.356

RR: Recirculation Ratio

⑤ Implications for energy efficiency and application

In order to commercialize an anaerobic process for wastewater treatment, it is important to consider the energy efficiency. The energy efficiency of the UASB reactor is estimated from the methane yield. However, in the case of the UABE reactor, both the electrical energy used and the methane production increase through the DIET are important factors for energy efficiency (Feng et al., 2016a; Song et al., 2016). The methane yield in the

UASB reactor treating acidic distillery wastewater was 195 mL/g COD_r, and the energy efficiency was only 46.9% (Table 6.4). However, the methane yield of the UABE reactor was 320 mL/g COD_r and the energy efficiency was 78%, which were considerably higher than those of the UASB reactor. The effluent recirculation ranging from 0.5 to 3.0 of the ratio to the influent flowrate significantly increased the energy efficiency of the UABE from 83.6 to 97.7 % (Table 6.4). The effluent recirculation increased the energy efficiency in the UASB reactor as well, but the improved energy efficiencies were always lower than those of the UABE. The efficiency of electron transfer from substrate to methane via DIET is higher than through IIET (Kouzuma et al., 2015; Rotaru et al., 2014a; Shen et al., 2016). The high energy efficiency in the UABE reactor is due to the high methane production through DIET compared to the UASB. The DIET for methane production in the anaerobic digester can be classified into bDIET, eDIET and cDIET (Fig. 6.12) (Kouzuma et al., 2015; Rotaru et al., 2014a; Shen et al., 2016; van Eerten-Jansen et al., 2015). In the UABE reactor, the methane portion of eDIET via electrode was only 2.8% (Table 6.4), and there was no conductive materials contributing to the cDIET in the influent acidic distillery wastewater. This indicates that the high energy efficiency of the UABE reactor was mainly attributed to the improved bDIET. The bDIET is electroactive bacteria directly transferring electrons to methane when the electroactive bacteria and methanogenic bacteria are in close proximity (Kouzuma et al., 2015; Rotaru et al., 2014b). These results indicate that electroactive bacteria in the UABE reactor are more enriched than in the UASB, and that the electroactive bacteria improve the DIET for methane production (van Eerten-Jansen et al., 2015; Zhao et al., 2016). In the UABE reactor, the results suggest that small electrical energy could enrich the electroactive bacteria in the suspended or granular state in the bulk solution, and the bDIET involved in the enriched electroactive bacteria greatly improves the energy efficiency of methane production (Fig. 6.12).

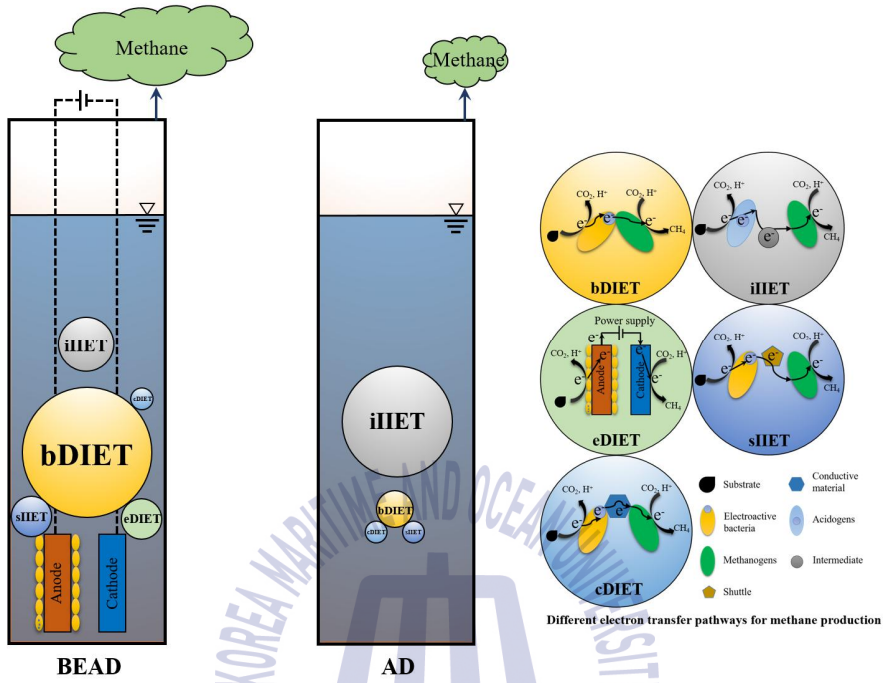


Fig. 6.12. Electron transfer pathways suggested for methane production in bioelectrochemical anaerobic digestion (BEAD) and anaerobic digestion (AD).

Biogas from anaerobic digestion requires an expensive purification process to increase the methane content, depending on the intended use (Appels et al., 2008; Luo & Angelidaki, 2012). It seems that the chemical composition of the substrate, but also the electron transfer pathway of the methanogenic reaction, generally determine the methane content of the biogas. The methane content of the biogas in the UABE and UASB reactors treating acidic distillery wastewater were 66.8% and 56.0%, respectively, but when the effluent recirculation ratio was increased to 3.0, the methane content increased to 83.0% and 70.1%, respectively (Table 6.4). This is attributed to the increase in methane production through the DIET pathway with high electron transfer efficiency by the effluent recirculation. The UABE reactor is a

process that is equipped with electrodes, and uses electrical energy during operation, indicating that the UABE reactor requires additional costs for initial installation and operation. However, despite the initial investment and operating costs, the high energy efficiency and high methane content of the biogas in the UABE reactor greatly increases profits. Therefore, this suggests that the UABE reactor is a high-rate anaerobic process with a very high potential of practical use for organic wastewater treatment.

6.4 Conclusions

Methane production and organic matter removal are considerably higher in an UABE reactor than in an UASB reactor when treating acidic distillery wastewater. In the UABE reactor, the enhancements of the eDIET and bDIET pathways for methane production were attributed to the enrichment of electroactive bacteria by a bioelectrochemical device. The partial neutralization of acidic distillery wastewater to pH 5.6 significantly enhanced eDIET and bDIET due to the improved activity of methanogenic and electroactive bacteria, as well as the IIET pathways for methane production. However, the electroactive bacteria are relatively less sensitive to a low pH compared to conventional anaerobic bacteria. Moreover, the effluent recirculation further enhances the bDIET for methane production in the UABE reactor, by neutralizing the influent acidity of the distillery wastewater, and increasing the upflow velocity. When the effluent recirculation ratio was 3.0 in the UABE, the methane production rate, and methane yield reached up to 3.88 L/L.d, and 501.0 mL/g COD_r, respectively. The UABE reactor is an excellent high-performance anaerobic process which can achieve high methane production and good organic removal efficiency suitable for the treatment of high-strength acidic wastewater.

Chapter 7: Conclusion and Further Study

7.1 Summary and conclusions

In this study, a bioelectrochemical anaerobic digester (BEAD) was developed for high-rate methane production. In order to achieve the purpose, modified anode and cathode were developed for the reactor. A BEAD reactor was used to treat sewage sludge by varying parameters, such as HRT, temperature and applied voltage. The electron transfer pathway for methane production in the BEAD was explored, and performance of an upflow anaerobic bioelectrochemical (UABE) reactor was evaluated with acidic distillery wastewater.

Graphite fiber fabric (GFF), modified using different methods, was studied as the anode and cathode for BEAD process. In the anode experiment, the accumulated methane production of all modified GFF anodes increased by 12-70% more than the control GFF anode in batch BEAD reactor. The GFF anodes were modified by adding a scaffold layer of expanded graphite (EG), and multiwalled carbon nanotube (MWCNT) after electrophoretic deposition (EPD) pretreatment, significantly improving bioelectrochemical methane production. The highest values for maximum methane production rate (47.4 mL CH_4/g COD.d), and methane yield (322.0 mL CH_4/g COD) were obtained from the anode which had coal tar pitch binder in the scaffolds. In the cathode experiment, EPD was used to mount MWCNT, and electron transfer assisting materials, such as Ni, Fe, or ammonia, onto the surface of GFF to enhance the growth of electroactive bacteria. The accumulated methane production of all modified GFF cathodes increased by 12-51% more than the GFF control cathode. The maximum methane production was obtained from the cathode decorated with MWCNT and Ni (44.8 mL CH_4/L.d), which was 57.2% higher than the control GFF cathode. The methane yield was as much as 326.3 mL CH_4/g COD_r when compared to 252.8 mL CH_4/g COD_r by the control cathode. The effectiveness of electron transfer assisting materials on

the cathode for bioelectrochemical methane production was in the order of Ni, Fe, and ammonia.

The performance of BEAD reactor used to treat sewage sludge, was explored at different HRTs ranging from 20 to 5 days, under a constant temperature of 35 ℃, and an applied voltage of 0.3V between anode and cathode. At 20 days HRT, the performance was considerably high as volatile solids (VS) reduction was 70.5%, while the specific methane production rate was 407mL CH_4/L.d, and the methane content was 76.9% in biogas. The VS reduction and the methane content in biogas slightly deteriorated as the HRT was lowered from 20 days to 5 days, while the methane production rate increased to 1,339 mL CH_4/L.d. The overall energy efficiencies for methane recovery were in the range of 69.1%-98.7%, and the maximum energy efficiency appeared at 10 days HRT. These results showed that the BEAD system could obtain much higher VS reduction and energy recovery with shorter HRTs than the conventional anaerobic digestion technology. The BEAD reactor was also studied at ambient temperature (25 ℃) and compared to studies at mesophilic condition (35 ℃). At 10 days HRT, state variables such as pH, alkalinity and VFAs in the mesophilic bioelectrochemical digester were very stable, and the VS removal efficiency was as high as 55.4%. The methane production and the methane content in biogas were 698.7 mL/L.d and 76.1%, respectively. After the temperature was downshifted to 25 ℃, the methane production rate was reduced to 87.7% of that at 35 ℃, and the methane content in biogas was slightly reduced to 73.3%. However, there were no considerable differences in VS removal and energy efficiency, indicating that ambient temperature is not a stringent condition for bioelectrochemical methane production. The influence of applied voltage on BEAD reactor was also studied at ambient temperature (25±2 ℃). The stability of the BEAD was considerably good in terms of pH, alkalinity and VFAs at 0.3V and 0.5V, but VFA accumulation occurred at 0.7V. The specific methane production rate (370 mL CH_4/L.d) was the highest at 0.3V, but the methane content (80.6%) in biogas and the methane yield (350 mL

CH_4/ g COD_r) were higher at 0.5V, significantly better than those at 0.7V. The VS removal efficiency was 64-66% at 0.3V and 0.5V, but only 31% at 0.7V. The dominant species of planktonic microbial communities was *Cloacamonas* at 0.3V and 0.5V, but the percentage of hydrolytic bacteria species such as *Saprospiraceae*, *Fimbriimonas*, and *Ottowia pentelensis* was much higher at 0.7V. Based on digestion performance and planktonic microbial communities the optimal applied voltage for BEAD process was 0.3-0.5V.

The electron transfer pathways for methane production was investigated in the batch BEAD reactor. It was found that the bioelectrochemical device coupled with anaerobic digestion reactor enriches electroactive bacteria in bulk solution, as well as on electrode surface. In the BEAD process, the electrons from a substrate are mainly transferred directly to methane biologically or via the electrode, and the electrons were transferred indirectly via intermediates (iIIET) including acetate, hydrogen/formate or endogenous electron shuttle (sIIET). The biological direct interspecies electron transfer (bDIET) via the planktonic electroactive bacteria was high and it played a vital role in electron transfer efficiency, but the electron transfer via the electrodes (eDIET) had some losses due to internal resistance of the bioelectrochemical device. Moreover, the planktonic anaerobic bacteria in the bulk solution enriched the electroactive bacteria, and the electroactive bacteria improved the electron recovery efficiency by the increasing the bDIET activity, as well as decreasing eDIET. Increasing planktonic anaerobic bacteria in the bulk solution is a good strategy for improving methane production rate, and methane yield in BEAD process.

The performance of an upflow anaerobic bioelectrochemical reactor (UABE) was examined with neutralized distillery wastewater, and the electron transfer pathways for methane production were evaluated and compared to those in an upflow anaerobic sludge blanket reactor (UASB). Methane production in the UABE reactor is approximately 70% higher than an UASB reactor, likely due to the enhanced DIET pathways for methane production by bioelectrochemical

enrichment of electroactive bacteria. The maximum methane production, and energy efficiency in the UABE reactor were 3.56 L/L.d, and 93.1%, respectively, which were obtained by after partial neutralization of acidic wastewater to pH 5.6. The DIET pathways are efficiently enhanced by the enrichment of electroactive bacteria through a bioelectrochemical device, and the electroactive bacteria are relatively less affected by a low pH compared to conventional anaerobic bacteria. The recirculation of effluent containing alkalinity neutralized the acidic influent, and increased the upflow velocity in both reactors, in addition to improving direct interspecies electron transfer in the UABE. When the effluent recirculation ratio was 3.0 in the UABE, the methane production rate, and methane yield reached up to 3.88 L/L.d, and 501.0 mL/g COD_r, respectively. The UABE requires electrode installation and electrical energy for operation, but the benefits from increased methane production are much higher. The UABE reactor is a highly practical process recommended for anaerobic treatment of high-strength acidic wastewater.

As a result, the bioelectrochemical device coupled with anaerobic digestion can greatly improve the digestion efficiency in terms of organic matter removal, methane production rate, and energy efficiency. The BEAD process is considered to be a technology that overcomes the disadvantages of conventional anaerobic digestion.

7.2 Suggestions for further study

In order to achieve high-rate methane production from organic matter, further research should be conducted in bioelectrochemical anaerobic digestion. The working mechanism, in particular, needs to be thoroughly investigated and understood. Thus, future studies on the current topic are recommended as follows:

1. The mutual effects of direct interspecies electron transfer (DIET) and indirect interspecies electron transfer (IIET) pathways for methane production should be further studied. The working mechanism of DIET and IIET pathways for methane production need to be fully studied and perfected.

2. The biologically direct interspecies electron transfer (bDIET), which is highly efficient in transferring electrons to form methane, when compared to other electron transfer pathways, needs to be further improved.

3. The electrode placement, electrode area, and configuration of a bioelectrochemical anaerobic digester require further research, with variations in wastewater disposal, organic loading rate, and other influent factors.

4. The optimal operation conditions for a bioelectrochemical anaerobic digest er are considerably unexplored for practical application in wastewater treatment. Therefore, the bioelectrochemical anaerobic digester should be further studied and examined, in particular by scaling up for practical application.

References

Abu-Orf M, Bowden G, Pfrang W, et al. Wastewater engineering: treatment and Resource recovery. McGraw Hill Education, 2014.

Akarsubasi A T, Ince O, Oz N A, et al. Evaluation of performance, acetoclastic methanogenic activity and archaeal composition of full-scale UASB reactors treating alcohol distillery wastewaters. Process Biochemistry, 2006, 41(1): 28-35.

Amatore C, Jutand A. Rates and Mechanisms of Electron Transfer/Nickel-Catalyzed Homocoupling and Carboxylation Reactions. An Electrochemical Approach[J]. Acta Chemica Scandinavica, 1990, 44: 755-764.

Anderson A B. Theory at the electrochemical interface: reversible potentials and potential-dependent activation energies. Electrochimica Acta, 2003, 48(25): 3743-3749.

Anderson G K, Yang G. Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. Water Environment Research, 1992, 64(1): 53-59.

Ando T, Izhar S, Tominaga H, et al. Ammonia-treated carbon-supported cobalt tungsten as fuel cell cathode catalyst. Electrochimica Acta, 2010, 55(8): 2614-2621.

Angelidaki I, Ellegaard L, Ahring B K. A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: focusing on ammonia inhibition. Biotechnology and Bioengineering, 1993, 42(2): 159-166.

Appels L, Baeyens J, Degrève J, et al. Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 2008, 34(6): 755-781.

Ariunbaatar J, Panico A, Esposito G, et al. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Applied Energy, 2014, 123: 143-156.

Astals S, Esteban-Gutiérrez M, Fernández-Arévalo T, et al. Anaerobic digestion of seven different sewage sludges: a biodegradability and modelling study. Water Research, 2013, 47(16): 6033-6043.

Balch W E, Fox G E, Magrum L J, et al. Methanogens: reevaluation of a unique biological group[J]. Microbiological Reviews, 1979, 43(2): 260.

Basu S, Mukherjee S, Kaushik A, et al. Integrated treatment of molasses distillery wastewater using microfiltration (MF). Journal of Environmental Management, 2015, 158: 55-60.

Baudler A, Riedl S, Schröder U. Long-term performance of primary and secondary electroactive biofilms using layered corrugated carbon electrodes. Frontiers in Energy Research, 2014, 2: 30.

Bengtsson S, Werker A, Welander T. Production of polyhydroxyalkanoates by glycogen accumulating organisms treating a paper mill wastewater. Water Science and Technology, 2008, 58(2): 323-330.

Blanchet E, Pécastaings S, Erable B, et al. Protons accumulation during anodic phase turned to advantage for oxygen reduction during cathodic phase in reversible bioelectrodes. Bioresource Technology, 2014, 173: 224-230.

Bolzonella D, Pavan P, Battistoni P, et al. Mesophilic anaerobic digestion of waste activated sludge: influence of the solid retention time in the wastewater treatment process. Process Biochemistry, 2005, 40(3): 1453-1460.

Cazier E A, Trably E, Steyer J P, et al. Biomass hydrolysis inhibition at high hydrogen partial pressure in solid-state anaerobic digestion. Bioresource Technology, 2015, 190: 106-113.

Cercado B, Cházaro-Ruiz L F, Ruiz V, et al. Biotic and abiotic

characterization of bioanodes formed on oxidized carbon electrodes as a basis to predict their performance. Biosensors and Bioelectronics, 2013, 50: 373-381.

Chen Y, Cheng J J, Creamer K S. Inhibition of anaerobic digestion process: a review. Bioresource Technology, 2008, 99(10): 4044-4064.

Chen Y, Jiang S, Yuan H, et al. Hydrolysis and acidification of waste activated sludge at different pHs. Water Research, 2007, 41(3): 683-689.

Chen Y, Xu Y, Chen L, et al. Microbial electrolysis cells with polyaniline/multi-walled carbon nanotube-modified biocathodes. Energy, 2015, 88: 377-384.

Cheng Q, Chen Z. The cause analysis of the incomplete semi-circle observed in high frequency region of EIS obtained from TEL-covered pure copper. International Journal of Electrochemical Science, 2013, 8(6): 8282-8290.

Cheng S, Logan B E. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. Electrochemistry Communications, 2007, 9(3): 492-496.

Cheng S, Xing D, Call D F, et al. Direct biological conversion of electrical current into methane by electromethanogenesis. Environmental Science and Technology, 2009, 43(10): 3953-3958.

Clauwaert P, Aelterman P, De Schamphelaire L, et al. Minimizing losses in bio-electrochemical systems: the road to applications. Applied Microbiology and Biotechnology, 2008, 79(6): 901-913.

Connaughton S, Collins G, O'Flaherty V. Psychrophilic and mesophilic anaerobic digestion of brewery effluent: a comparative study. Water Research, 2006, 40(13): 2503-2510.

Corazza F C, Calsavara L P V, Moraes F F, et al. Determination of inhibition in the enzymatic hydrolysis of cellobiose using hybrid neural modeling. Brazilian Journal of Chemical Engineering, 2005, 22(1): 19-29.

Cuzin N, Ouattara A S, Labat M, et al. Methanobacterium congolense sp. nov., from a methanogenic fermentation of cassava peel. International Journal of Systematic and Evolutionary Microbiology, 2001, 51(2): 489-493.

Dai H, Yang H, Liu X, et al. Electrochemical evaluation of nano-Mg (OH) 2/graphene as a catalyst for hydrogen evolution in microbial electrolysis cell. Fuel, 2016, 174: 251-256.

Demitry M E, McFarland M J. Defining full-scale Anaerobic Digestion Stability: The Case of Central Weber Sewer Improvement District. Environment and Pollution, 2015, 4(2): 1.

Deng Q, Li X, Zuo J, et al. Power generation using an activated carbon fiber felt cathode in an upflow microbial fuel cell. Journal of Power Sources, 2010, 195(4): 1130-1135.

Diaz C, Cortizo M C, Schilardi P L, et al. Influence of the nano-micro structure of the surface on bacterial adhesion. Materials Research, 2007, 10(1): 11-14.

Dube C D, Guiot S R. Direct interspecies electron transfer in anaerobic digestion: a review. Biogas Science and Technology. Springer International Publishing, 2015: 101-115.

Esposito G, Frunzo L, Giordano A, et al. Anaerobic co-digestion of organic wastes. Reviews in Environmental Science and Bio/Technology, 2012, 11(4): 325-341.

Feng Q, Song Y C, Bae B U. Influence of applied voltage on the performance of bioelectrochemical anaerobic digestion of sewage sludge and planktonic microbial communities at ambient temperature. Bioresource Technology, 2016a, 220: 500-508.

Feng Q, Song Y C, Yoo K, et al. Performance of upflow anaerobic bioelectrochemical reactor compared to the sludge blanket reactor for acidic distillery wastewater treatment. Journal of Korean Society of

Environmental Engineers, 2016b, 38(6): 279-290.

Feng Q, Song Y C, Yoo K, et al. Influence of neutralization in acidic distillery wastewater on direct interspecies electron transfer for methane production in an upflow anaerobic bioelectrochemical reactor", International Journal of Hydrogen Energy, 2017a, accepted.

Feng Q, Song Y C, Yoo K, et al. Bioelectrochemical enhancement of direct interspecies electron transfer in upflow anaerobic reactor with effluent recirculation for acidic distillery wastewater. Bioresource Technology, 2017b, 241(8): 171-180.

Feng Q, Song Y C. Surface modification of a graphite fiber fabric anode for enhanced bioelectrochemical methane production. Energy and Fuels, 2016a, 30(8): 6467-6474.

Feng Q, Song Y C. Decoration of graphite fiber fabric cathode with electron transfer assisting material for enhanced bioelectrochemical methane production. Journal of Applied Electrochemistry, 2016b, 46(12): 1211-1219.

Feng Y, Zhang Y, Chen S, et al. Enhanced production of methane from waste activated sludge by the combination of high-solid anaerobic digestion and microbial electrolysis cell with iron–graphite electrode. Chemical Engineering Journal, 2015, 259: 787-794.

Gajaraj S, Huang Y, Zheng P, et al. Methane production improvement and associated methanogenic assemblages in bioelectrochemically assisted anaerobic digestion. Biochemical Engineering Journal, 2017, 117: 105-112.

Garrigues C, Loubiere P, Lindley N D, et al. Control of the shift from homolactic acid to mixed-acid fermentation in Lactococcus lactis: predominant role of the NADH/NAD^+ ratio. Journal of Bacteriology, 1997, 179(17): 5282-5287.

Gavala H N, Yenal U, Skiadas I V, et al. Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of

pre-treatment at elevated temperature. Water Research, 2003, 37(19): 4561-4572.

Ghaly A E. A comparative study of anaerobic digestion of acid cheese whey and dairy manure in a two-stage reactor. Bioresource Technology, 1996, 58(1): 61-72.

Ghasemi M, Daud W R W, Hassan S H A, et al. Nano-structured carbon as electrode material in microbial fuel cells: a comprehensive review. Journal of Alloys and Compounds, 2013, 580: 245-255.

Gomez X, Cuetos M J, Cara J, et al. Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes: conditions for mixing and evaluation of the organic loading rate. Renewable Energy, 2006, 31(12): 2017-2024.

Guerrero L, Omil F, Mendez R, et al. Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein. Water Research, 1999, 33(15): 3281-3290.

Guo X, Liu J, Xiao B. Bioelectrochemical enhancement of hydrogen and methane production from the anaerobic digestion of sewage sludge in single-chamber membrane-free microbial electrolysis cells. International Journal of Hydrogen Energy, 2013, 38(3): 1342-1347.

Gutierrez O, Park D, Sharma K R, et al. Effects of long-term pH elevation on the sulfate-reducing and methanogenic activities of anaerobic sewer biofilms. Water Research, 2009, 43(9): 2549-2557.

Habeeb S A, Latiff A, Daud Z B, et al. A review on granules initiation and development inside UASB reactor and the main factors affecting granules formation process. International Journal of Energy and Environment, 2011, 2(2): 311-320.

Hamelers H V M, Ter Heijne A, Sleutels T H J A, et al. New applications and performance of bioelectrochemical systems. Applied Microbiology and Biotechnology, 2010, 85(6): 1673-1685.

Harada H, Uemura S, Chen A C, et al. Anaerobic treatment of a recalcitrant distillery wastewater by a thermophilic UASB reactor. Bioresource Technology, 1996, 55(3): 215-221.

Heidrich E S, Edwards S R, Dolfing J, et al. Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12month period. Bioresource Technology, 2014, 173: 87-95.

Hou J, Song W, Yu H, et al. Electrochemical impedance investigation of proton exchange membrane fuel cells experienced subzero temperature. Journal of Power Sources, 2007, 171(2): 610-616.

Hu H, Fan Y, Liu H. Hydrogen production using single-chamber membrane-free microbial electrolysis cells. Water Research, 2008, 42(15): 4172-4178.

Ikumi D S, Harding T H, Ekama G A. Biodegradability of wastewater and activated sludge organics in anaerobic digestion. Water Research, 2014, 56: 267-279.

Ishihara K, Nguyen P A, Groen A C, et al. Microtubule nucleation remote from centrosomes may explain how asters span large cells. Proceeding of the National Academy of Sciences, 2014, 111(50): 17715-17722.

Jiang Y, Su M, Zhang Y, et al. Bioelectrochemical systems for simultaneously production of methane and acetate from carbon dioxide at relatively high rate. International Journal of Hydrogen Energy, 2013, 38(8): 3497-3502.

Juste-Poinapen NMS, Turner MS, Rabaey K, et al. Evaluating the potential impact of proton carriers on syntrophic propionate oxidation. Scientific Reports, 2015, 5: 18364.

Kadier A, Simayi Y, Chandrasekhar K, et al. Hydrogen gas production with an electroformed Ni mesh cathode catalysts in a single-chamber microbial electrolysis cell (MEC). International Journal of Hydrogen Energy, 2015,

40(41): 14095-14103.

Kadier A, Simayi Y, Kalil M S, et al. A review of the substrates used in microbial electrolysis cells (MECs) for producing sustainable and clean hydrogen gas. Renewable Energy, 2014, 71: 466-472.

Kang H, Korea Country Report, Proceeding of IEA Bioenergy, Berlin (Germany), 2015.

Kalakonda P, Cabrera Y, Judith R, et al. Studies of electrical and thermal conductivities of sheared multi-walled carbon nanotube with isotactic polypropylene polymer composites. Nanomaterials and Nanotechnology, 2015, 5: 2.

Kalyuzhnyi S V, Fedorovich V V, Lens P. Dispersed plug flow model for upflow anaerobic sludge bed reactors with focus on granular sludge dynamics. Journal of Industrial Microbiology and Biotechnology, 2006, 33(3): 221-237.

Karakashev D, Batstone D J, Angelidaki I. Influence of environmental conditions on methanogenic compositions in anaerobic biogas reactors. Applied and Environmental Microbiology, 2005, 71(1): 331-338.

Kardos L, Juhasz A, Palko G, et al. Comparing of mesophilic and thermophilic anaerobic fermented sewage sludge based on chemical and biochemical tests. Applied Ecology and Environmental Research, 2011, 9(3): 293-302.

Kato S. Biotechnological aspects of microbial extracellular electron transfer. Microbes and environments, 2015, 30(2): 133-139.

Katsikogianni M, Missirlis Y F. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. European Cells and Materials, 2004, 8(3).

Kaviyarasan K. Application of UASB Reactor in Industrial Wastewater Treatment–A Review. International Journal of Scientific and Engineering Research, 2014, 5(1): 584.

Kaya C, Kaya F, Su B, et al. Structural and functional thick ceramic coatings by electrophoretic deposition. Surface and Coatings Technology, 2005, 191(2): 303-310.

Khalid A, Arshad M, Anjum M, et al. The anaerobic digestion of solid organic waste. Waste Management, 2011, 31(8): 1737-1744.

Kim J R, Song Y E, Munussami G, et al. Recent applications of bioelectrochemical system for useful resource recovery: Retrieval of nutrient and metal from wastewater. Geosystem Engineering, 2015a, 18(4): 173-180.

Kim D H, Song Y C, Qing F. Influence of applied voltage for bioelectrochemical anaerobic digestion of sewage sludge. Journal of Korean Society of Environmental Engineers, 2015b, 37(9): 542-549.

Kim J, Park C, Kim T H, et al. Effects of various pretreatments for enhanced anaerobic digestion with waste activated sludge. Journal of Bioscience and Bioengineering, 2003, 95(3): 271-275.

Kim K S, Park S J. Bridge effect of carbon nanotubes on the electrical properties of expanded graphite/poly (ethylene terephthalate) nanocomposites. Carbon Letters, 2012, 13(1): 51-55.

Kjelleberg S, Albertson N, Flärdh K, et al. How do non-differentiating bacteria adapt to starvation. Antonie van Leeuwenhoek, 1993, 63(3): 333-341.

Koch C, Kuchenbuch A, Kretzschmar J, et al. Coupling electric energy and biogas production in anaerobic digesters–impacts on the microbiome. RSC Advances, 2015, 5(40): 31329-31340.

Komemoto K, Lim Y G, Nagao N, et al. Effect of temperature on VFA's and biogas production in anaerobic solubilization of food waste. Waste Management, 2009, 29(12): 2950-2955.

Kondaveeti S, Min B. Bioelectrochemical reduction of volatile fatty acids in anaerobic digestion effluent for the production of biofuels. Water

Research, 2015, 87: 137-144.

Koster I W, Cramer A. Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. Applied and Environmental Microbiology, 1987, 53(2): 403-409.

Kouzuma A, Kato S, Watanabe K. Microbial interspecies interactions: recent findings in syntrophic consortia. Frontiers in Microbiology, 2015, 6: 477.

Kumar G, Saratale R G, Kadier A, et al. A review on bio-electrochemical systems (BESs) for the syngas and value added biochemicals production. Chemosphere, 2017, 177: 84-92.

Kumar R, Kant R. Generalized Warburg impedance on realistic self-affine fractals: Comparative study of statistically corrugated and isotropic roughness. Journal of Chemical Sciences, 2009, 121(5): 579.

Labatut R A, Angenent L T, Scott N R. Conventional mesophilic vs. thermophilic anaerobic digestion: a trade-off between performance and stability. Water Research, 2014, 53: 249-258.

Laidler K J. The development of the Arrhenius equation. Journal of Chemical Education, 1984, 61(6): 494.

Larrosa-Guerrero A, Scott K, Head I M, et al. Effect of temperature on the performance of microbial fuel cells. Fuel, 2010, 89(12): 3985-3994.

Lee C Y, Park S H, Song Y C, et al. Effects of anode surface area and methylene blue dye treatment on the power density of microbial fuel cell with sponge and carbon nano tube electrode. Journal of Korean Society of Water and Wastewater Vol, 2012, 26(6): 883-888.

Li Y, Zhang Y, Liu Y, et al. Enhancement of anaerobic methanogenesis at a short hydraulic retention time via bioelectrochemical enrichment of hydrogenotrophic methanogens. Bioresource Technology, 2016, 218: 505-511.

Liang D W, Peng S K, Lu S F, et al. Enhancement of hydrogen production

in a single chamber microbial electrolysis cell through anode arrangement optimization. Bioresource Technology, 2011, 102(23): 10881-10885.

Liu D, Zhang L, Chen S, et al. Bioelectrochemical enhancement of methane production in low temperature anaerobic digestion at 10℃. Water Research, 2016, 99: 281-287.

Liu G, Li X, Ganesan P, et al. Studies of oxygen reduction reaction active sites and stability of nitrogen-modified carbon composite catalysts for PEM fuel cells. Electrochimica Acta, 2010, 55(8): 2853-2858.

Liu W, Cheng S, Guo J. Anode modification with formic acid: A simple and effective method to improve the power generation of microbial fuel cells. Applied Surface Science, 2014, 320: 281-286.

Liu W, Huang S, Zhou A, et al. Hydrogen generation in microbial electrolysis cell feeding with fermentation liquid of waste activated sludge. International Journal of Hydrogen Energy, 2012, 37(18): 13859-13864.

Logan B E, Call D, Cheng S, et al. Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environmental Science and Technology, 2008, 42(23): 8630-8640.

Lovley DR. Powering microbes with electricity: direct electron transfer from electrodes to microbes. Environmental Microbiology Reports, 2011, 3(1): 27-35.

Lueders T, Chin K J, Conrad R, et al. Molecular analyses of methyl-coenzyme M reductase α-subunit (mcrA) genes in rice field soil and enrichment cultures reveal the methanogenic phenotype of a novel archaeal lineage. Environmental Microbiology, 2001, 3(3): 194-204.

Luo G, Angelidaki I. Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture. Biotechnology and Bioengineering, 2012a, 109(11): 2729-2736.

Luo J, Li M, Zhou M, et al. Characterization of a novel strain

phylogenetically related to Kocuria rhizophila and its chemical modification to improve performance of microbial fuel cells. Biosensors and Bioelectronics, 2015, 69: 113-120.

Luo K, Yang Q, Li X, et al. Hydrolysis kinetics in anaerobic digestion of waste activated sludge enhanced by α-amylase. Biochemical Engineering Journal, 2012b, 62: 17-21.

Lyberatos G, Skiadas I V. Modelling of anaerobic digestion–a review. Global Nest International Journal, 1999, 1(2): 63-76.

Mansfeld F. Tafel slopes and corrosion rates obtained in the pre-Tafel region of polarization curves. Corrosion Science, 2005, 47(12): 3178-3186.

Mao C, Feng Y, Wang X, et al. Review on research achievements of biogas from anaerobic digestion. Renewable and Sustainable Energy Reviews, 2015, 45: 540-555.

Marsili E, Baron D B, Shikhare I D, et al. Shewanella secretes flavins that mediate extracellular electron transfer. Proceedings of the National Academy of Sciences, 2008, 105(10): 3968-3973.

Martin W F, Neukirchen S, Zimorski V, et al. Energy for two: New archaeal lineages and the origin of mitochondria. BioEssays, 2016, 38(9): 850-856.

Martinez-Perinan E, Gennari M, Revenga-Parra M, et al. Highly dense nickel hydroxide nanoparticles catalyst electrodeposited from a novel Ni (II) paddle–wheel complex. Journal of Catalysis, 2015, 329: 22-31.

Masse D I, Saady N M C. High rate psychrophilic anaerobic digestion of undiluted dairy cow feces. Bioresource technology, 2015, 187: 128-135.

Mata-Alvarez J. Anaerobic digestion of the organic fraction of municipal solid waste: a perspective. Biomethanization of the organic fraction of municipal solid wastes, 2003: 91-110.

Mata-Alvarez J, Dosta J, Romero-Güiza M S, et al. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable

and Sustainable Energy Reviews, 2014, 36: 412-427.

McCarty P L. Environmental biotechnology: principles and applications. Tata McGraw-Hill Education, 2012.

Mir M A, Hussain A, Verma C. Design considerations and operational performance of anaerobic digester: A review. Cogent Engineering, 2016, 3(1): 1181696.

Mohana S, Acharya B K, Madamwar D. Distillery spent wash: Treatment technologies and potential applications. Journal of Hazardous Materials, 2009, 163(1): 12-25.

Mottet A, François E, Latrille E, et al. Estimating anaerobic biodegradability indicators for waste activated sludge. Chemical Engineering Journal, 2010, 160(2): 488-496.

Nam J Y, Tokash J C, Logan B E. Comparison of microbial electrolysis cells operated with added voltage or by setting the anode potential. International Journal of Hydrogen Energy, 2011, 36(17): 10550-10556.

Oliveira V B, Simões M, Melo L F, et al. Overview on the developments of microbial fuel cells. Biochemical Engineering Journal, 2013, 73: 53-64.

Ostrem K, Themelis NJ. Greening waste: Anaerobic digestion for treating the organic fraction of municipal solid wastes. Earth Engineering Center, Columbia University, 2004: 6-9.

Padilla-Gasca E, López-López A, Gallardo-Valdez J. Evaluation of stability factors in the anaerobic treatment of slaughterhouse wastewater. Journal of Bioremediation and Biodegradation, 2011, 2: 114.

Pan Y, Yang N, Chen Y, et al. Nickel phosphide nanoparticles-nitrogen-doped graphene hybrid as an efficient catalyst for enhanced hydrogen evolution activity. Journal of Power Sources, 2015, 297: 45-52.

Pant D, Adholeya A. Biological approaches for treatment of distillery wastewater: a review. Bioresource Technology, 2007, 98(12): 2321-2334.

Park J K, Do I H, Askeland P, et al. Electrodeposition of exfoliated graphite nanoplatelets onto carbon fibers and properties of their epoxy composites. Composites Science and Technology, 2008, 68(7): 1734-1741.

Parot S, Délia M L, Bergel A. Forming electroactive biofilms from garden compost under chronoamperometry. Bioresource Technology, 2008, 99(11): 4809-4816.

Peces M, Astals S, Mata-Alvarez J. Response of a sewage sludge mesophilic anaerobic digester to short and long-term thermophilic temperature fluctuations. Chemical Engineering Journal, 2013, 233: 109-116.

Pelletier E, Kreimeyer A, Bocs S, et al. "Candidatus Cloacamonas acidaminovorans": genome sequence reconstruction provides a first glimpse of a new bacterial division. Journal of Bacteriology, 2008, 190(7): 2572-2579.

Pereira L, Pereira R, Pereira M F R, et al. Effect of different carbon materials as electron shuttles in the anaerobic biotransformation of nitroanilines. Biotechnology and Bioengineering, 2016, 113(6): 1194-1202.

Protsenko V S, Danilov F I. Activation energy of electrochemical reaction measured at a constant value of electrode potential. Journal of Electroanalytical Chemistry, 2011, 651(2): 105-110.

Quintero O M S, Chaparro W A, Ipaz L, et al. Influence of the microstructure on the electrochemical properties of Al-Cr-N coatings deposited by co-sputtering method from a Cr-Al binary target. Materials Research, 2013, 16(1): 204-214.

Rabaey K, Rozendal R A. Microbial electrosynthesis—revisiting the electrical route for microbial production. Nature Reviews Microbiology, 2010, 8(10): 706-716.

Rader G K, Logan B E. Multi-electrode continuous flow microbial electrolysis cell for biogas production from acetate. International Journal of Hydrogen Energy, 2010, 35(17): 8848-8854.

Rahimnejad M, Adhami A, Darvari S, et al. Microbial fuel cell as new technology for bioelectricity generation: A review. Alexandria Engineering Journal, 2015, 54(3): 745-756.

Ran Z, Gefu Z, Kumar J A, et al. Hydrogen and methane production in a bio-electrochemical system assisted anaerobic baffled reactor. International Journal of Hydrogen Energy, 2014, 39(25): 13498-13504.

Reeve H A, Lauterbach L, Lenz O, et al. Enzyme-Modified Particles for Selective Biocatalytic Hydrogenation by Hydrogen-Driven NADH Recycling. ChemCatChem, 2015, 7(21): 3480-3487.

Regueiro L, Carballa M, Lema J M. Outlining microbial community dynamics during temperature drop and subsequent recovery period in anaerobic co-digestion systems. Journal of Biotechnology, 2014, 192: 179-186.

Ren N, Zhao D, Chen X, et al. Mechanism and controlling strategy of the production and accumulation of propionic acid for anaerobic wastewater treatment. Science in China Series B: Chemistry, 2002, 45(3): 319-327.

Reungsang A, Sittijunda S, Sreela-or C. Methane production from acidic effluent discharged after the hydrogen fermentation of sugarcane juice using batch fermentation and UASB reactor. Renewable Energy, 2016, 86: 1224-1231.

Richter K, Gescher J. Accelerated glycerol fermentation in Escherichia coli using methanogenic formate consumption. Bioresource Technology, 2014, 162: 389-391.

Richter K, Schicklberger M, Gescher J. Dissimilatory reduction of extracellular electron acceptors in anaerobic respiration. Applied and Environmental Microbiology, 2012, 78(4): 913-921.

Ritmann B E, McCarty P L. Environmental biotechnology: principles and applications. 10020. New York NY: MacGraw-Hill, 2001.

Rolfe M D, Rice C J, Lucchini S, et al. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient

metal accumulation. Journal of Bacteriology, 2012, 194(3): 686-701.

Rose M A, Williamson M A, Willit J. Determining the exchange current density and Tafel constant for uranium in LiCl/KCl eutectic. ECS Electrochemistry Letters, 2015, 4(1): 5-7.

Rotaru A E, Shrestha P M, Liu F, et al. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. Energy and Environmental Science, 2014a, 7(1): 408-415.

Rotaru A E, Shrestha P M, Liu F, et al. Direct interspecies electron transfer between Geobacter metallireducens and Methanosarcina barkeri. Applied and Environmental Microbiology, 2014b, 80(15): 4599-4605.

Rubia D L, Perez M, Romero L I, et al. Anaerobic mesophilic and thermophilic municipal sludge digestion. Chemical and Biochemical Engineering Quarterly, 2002, 16(3): 119-124.

Sakai H, Nakagawa T, Tokita Y, et al. A high-power glucose/oxygen biofuel cell operating under quiescent conditions. Energy and Environmental Science, 2009, 2(1): 133-138.

Samsudeen N, Radhakrishnan T K, Matheswaran M. Effect of isolated bacterial strains from distillery wastewater on power generation in microbial fuel cell. Process Biochemistry, 2016, 51(11): 1876-1884.

Sangeetha T, Guo Z, Liu W, et al. Cathode material as an influencing factor on beer wastewater treatment and methane production in a novel integrated upflow microbial electrolysis cell (Upflow-MEC). International Journal of Hydrogen Energy, 2016, 41(4): 2189-2196.

Sasaki D, Sasaki K, Watanabe A, et al. Operation of a cylindrical bioelectrochemical reactor containing carbon fiber fabric for efficient methane fermentation from thickened sewage sludge. Bioresource Technology, 2013, 129: 366-373.

Sasaki K, Morita M, Sasaki D, et al. A bioelectrochemical reactor containing

carbon fiber textiles enables efficient methane fermentation from garbage slurry. Bioresource Technology, 2011, 102(13): 6837-6842.

Sato K, Kawaguchi H, Kobayashi H. Bio-electrochemical conversion of carbon dioxide to methane in geological storage reservoirs. Energy Conversion and Management, 2013, 66: 343-350.

Shen L, Zhao Q, Wu X, et al. Interspecies electron transfer in syntrophic methanogenic consortia: From cultures to bioreactors. Renewable and Sustainable Energy Reviews, 2016, 54: 1358-1367.

Shin H S, Song Y C. A model for evaluation of anaerobic degradation characteristics of organic waste: focusing on kinetics, rate-limiting step. Environmental Technology, 1995, 16(8): 775-784.

Shrestha P M, Malvankar N S, Werner J J, et al. Correlation between microbial community and granule conductivity in anaerobic bioreactors for brewery wastewater treatment. Bioresource Technology, 2014, 174: 306-310.

Shrestha P M, Rotaru A E. Plugging in or going wireless: strategies for interspecies electron transfer. Intra-and inter-species interactions in microbial communities, 2015, 142.

Sieber J R, McInerney M J, Gunsalus R P. Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. Annual Review of Microbiology, 2012, 66: 429-452.

Siegert M, Yates M D, Call D F, et al. Comparison of nonprecious metal cathode materials for methane production by electromethanogenesis. ACS Sustainable Chemistry and Engineering, 2014, 2(4): 910-917.

Silvestre G, Bonmatí A, Fernández B. Optimisation of sewage sludge anaerobic digestion through co-digestion with OFMSW: effect of collection system and particle size. Waste Management, 2015, 43: 137-143.

Singh S, Verma N. Graphitic carbon micronanofibers asymmetrically dispersed

with alumina-nickel nanoparticles: a novel electrode for mediatorless microbial fuel cells. International Journal of Hydrogen Energy, 2015, 40(17): 5928-5938.

Smith P H, Mah R A. Kinetics of acetate metabolism during sludge digestion. Applied Microbiology, 1966, 14(3): 368-371.

Song Y C, Feng Q, Ahn Y. Performance of the bio-electrochemical anaerobic digestion of sewage sludge at different hydraulic retention times. Energy and Fuels, 2016, 30(1): 352-359.

Song Y C, Kim D S, Woo J H, et al. Effect of surface modification of anode with surfactant on the performance of microbial fuel cell. International Journal of Energy Research, 2015a, 39(6): 860-868.

Song Y C, Kim D S, Woo J H. Effect of epoxy mixed with nafion solution as an anode binder on the performance of microbial fuel cell. Journal of Korean Society of Environmental Engineers, 2014, 36(1): 1-6.

Song Y C, Kim D S, Woo J H. Effect of Multiwall Carbon Nanotube contained in the Exfoliated Graphite anode on the power production and internal resistance of microbial fuel cells. KSCE Journal of Civil Engineering, 2015b, 19(4): 857-863.

Song Y C, Kwon S J, Woo J H. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic-and thermophilic digestion of sewage sludge. Water Research, 2004, 38(7): 1653-1662.

Souza M E, Fuzaro G, Polegato A R. Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. Water Science and Technology, 1992, 25(7): 213-222.

Strauber H, Schröder M, Kleinsteuber S. Metabolic and microbial community dynamics during the hydrolytic and acidogenic fermentation in a leach-bed process. Energy, Sustainability and Society, 2012, 2(1): 13.

Sun J, Hu Y, Hou B. Electrochemical characteriztion of the bioanode during

simultaneous azo dye decolorization and bioelectricity generation in an air-cathode single chambered microbial fuel cell. Electrochimica Acta, 2011, 56(19): 6874-6879.

Sun R, Zhou A, Jia J, et al. Characterization of methane production and microbial community shifts during waste activated sludge degradation in microbial electrolysis cells. Bioresource Technology, 2015, 175: 68-74.

Sunada N S, Orrico A C A, Júnior O, et al. Potential of biogas and methane production from anaerobic digestion of poultry slaughterhouse effluent. Revista Brasileira de Zootecnia, 2012, 41(11): 2379-2383.

Takashima M, Tanaka Y. Acidic thermal post-treatment for enhancing anaerobic digestion of sewage sludge. Journal of Environmental Chemical Engineering, 2014, 2(2): 773-779.

Tartakovsky B, Manuel M F, Wang H, et al. High rate membrane-less microbial electrolysis cell for continuous hydrogen production. International Journal of Hydrogen Energy, 2009, 34(2): 672-677.

Tang J, Chen S, Yuan Y, et al. In situ formation of graphene layers on graphite surfaces for efficient anodes of microbial fuel cells. Biosensors and Bioelectronics, 2015, 71: 387-395.

Thiele J H, Chartrain M, Zeikus J G. Control of interspecies electron flow during anaerobic digestion: role of floc formation in syntrophic methanogenesis. Applied and Environmental Microbiology, 1988, 54(1): 10-19.

Tian Y, Ji C, Wang K, et al. Assessment of an anaerobic membrane bio-electrochemical reactor (AnMBER) for wastewater treatment and energy recovery. Journal of Membrane Science, 2014, 450: 242-248.

Tsai H Y, Wu C C, Lee C Y, et al. Microbial fuel cell performance of multiwall carbon nanotubes on carbon cloth as electrodes. Journal of Power Sources, 2009, 194(1): 199-205.

Ueki A, Akasaka H, Suzuki D, et al. Paludibacter propionicigenes gen. nov.,

sp. nov., a novel strictly anaerobic, Gram-negative, propionate-producing bacterium isolated from plant residue in irrigated rice-field soil in Japan. International Journal of Systematic and Evolutionary Microbiology, 2006, 56(1): 39-44.

Ullery M L, Logan B E. Anode acclimation methods and their impact on microbial electrolysis cells treating fermentation effluent. International Journal of Hydrogen Energy, 2015, 40(21): 6782-6791.

van der Zee F P, Cervantes F J. Impact and application of electron shuttles on the redox (bio) transformation of contaminants: a review. Biotechnology Advances, 2009, 27(3): 256-277.

van Eerten-Jansen M C A A, Jansen N C, Plugge C M, et al. Analysis of the mechanisms of bioelectrochemical methane production by mixed cultures. Journal of Chemical Technology and Biotechnology, 2015, 90(5): 963-970.

van Gestel N C, Reischke S, Bååth E. Temperature sensitivity of bacterial growth in a hot desert soil with large temperature fluctuations. Soil Biology and Biochemistry, 2013, 65: 180-185.

Vandecandelaere I, Nercessian O, Faimali M, et al. Bacterial diversity of the cultivable fraction of a marine electroactive biofilm. Bioelectrochemistry, 2010, 78(1): 62-66.

Varel V H, Hashimoto A G, Chen Y R. Effect of temperature and retention time on methane production from beef cattle waste. Applied and Environmental Microbiology, 1980, 40(2): 217-222.

Vasquez J, Nakasaki K. Effects of shock loading versus stepwise acclimation on microbial consortia during the anaerobic digestion of glycerol. Biomass and Bioenergy, 2016, 86: 129-135.

Villano M, Aulenta F, Ciucci C, et al. Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. Bioresource Technology, 2010,

101(9): 3085-3090.

Villano M, Monaco G, Aulenta F, et al. Electrochemically assisted methane production in a biofilm reactor. Journal of Power Sources, 2011, 196(22): 9467-9472.

Villano M, Ralo C, Zeppilli M, et al. Influence of the set anode potential on the performance and internal energy losses of a methane-producing microbial electrolysis cell. Bioelectrochemistry, 2016, 107: 1-6.

Villano M, Scardala S, Aulenta F, et al. Carbon and nitrogen removal and enhanced methane production in a microbial electrolysis cell. Bioresource Technology, 2013, 130: 366-371.

Walker M, Zhang Y, Heaven S, et al. Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. Bioresource Technology, 2009, 100(24): 6339-6346.

Wang A, Liu W, Cheng S, et al. Source of methane and methods to control its formation in single chamber microbial electrolysis cells. International Journal of Hydrogen Energy, 2009, 34(9): 3653-3658.

Wang D, Han H, Han Y, et al. Enhanced treatment of Fischer-Tropsch (FT) wastewater using the up-flow anaerobic sludge blanket coupled with bioelectrochemical system: Effect of electric field. Bioresource Technology, 2017, 232: 18-26.

Wang Y J, Fang B, Li H, et al. Progress in modified carbon support materials for Pt and Pt-alloy cathode catalysts in polymer electrolyte membrane fuel cells. Progress in Materials Science, 2016, 82: 445-498.

Wang Z W, Ma J, Chen S. Bipolar effects of settling time on active biomass retention in anaerobic sequencing batch reactors digesting flushed dairy manure. Bioresource Technology, 2011, 102(2): 697-702.

Watson V J, Delgado C N, Logan B E. Improvement of activated carbons as oxygen reduction catalysts in neutral solutions by ammonia gas treatment and their performance in microbial fuel cells. Journal of Power Sources,

2013, 242: 756-761.

Wei J, Liang P, Huang X. Recent progress in electrodes for microbial fuel cells. Bioresource Technology, 2011, 102(20): 9335-9344.

Woo J H, Song Y C. Influence of temperature and duration of heat treatment used for anaerobic seed sludge on biohydrogen fermentation. KSCE Journal of Civil Engineering, 2010, 14(2): 141-147.

Wu B, Feng C, Huang L, et al. Anode-biofilm electron transfer behavior and wastewater treatment under different operational modes of bioelectrochemical system. Bioresource Technology, 2014a, 157: 305-309.

Wu C, Cheng Y Y, Li B B, et al. Electron acceptor dependence of electron shuttle secretion and extracellular electron transfer by Shewanella oneidensis MR-1. Bioresource Technology, 2013, 136: 711-714.

Wu W, Lesnik K L, Xu S, et al. Impact of tobramycin on the performance of microbial fuel cell. Microbial Cell Factories, 2014b, 13(1): 91.

Xafenias N, Mapelli V. Performance and bacterial enrichment of bioelectrochemical systems during methane and acetate production. International Journal of Hydrogen Energy, 2014, 39(36): 21864-21875.

Yamada Y, Iriyama Y, Abe T, et al. Kinetics of electrochemical insertion and extraction of lithium ion at SiO. Journal of The Electrochemical Society, 2010, 157(1): A26-A30.

Yang L, Huang Y, Zhao M, et al. Enhancing biogas generation performance from food wastes by high-solids thermophilic anaerobic digestion: Effect of pH adjustment. International Biodeterioration and Biodegradation, 2015, 105: 153-159.

Yang Y, Xu M, Guo J, et al. Bacterial extracellular electron transfer in bioelectrochemical systems. Process Biochemistry, 2012, 47(12): 1707-1714.

Yen S J, Tsai M C, Wang Z C, et al. The improvement of catalytic

efficiency by optimizing Pt on carbon cloth as a cathode of a microbial fuel cell. Electrochimica Acta, 2013, 108: 241-247.

Yoon S M, Choi C H, Kim M, et al. Enrichment of electroactive bacteria using a three-electrode electrochemical cell. Journal of Microbiology and Biotechnology, 2007, 17(1): 110-115.

Yu Y Y, Guo C X, Yong Y C, et al. Nitrogen doped carbon nanoparticles enhanced extracellular electron transfer for high-performance microbial fuel cells anode. Chemosphere, 2015, 140: 26-33.

Yuan H, Zhu N. Progress in inhibition mechanisms and process control of intermediates and by-products in sewage sludge anaerobic digestion. Renewable and Sustainable Energy Reviews, 2016, 58: 429-438.

Yuan Y, Zhao B, Zhou S, et al. Electrocatalytic activity of anodic biofilm responses to pH changes in microbial fuel cells. Bioresource Technology, 2011, 102(13): 6887-6891.

Zhang D, Chen Y, Zhao Y, et al. New sludge pretreatment method to improve methane production in waste activated sludge digestion. Environmental Science and Technology, 2010, 44(12): 4802-4808.

Zhang F, Xia X, Luo Y, et al. Improving startup performance with carbon mesh anodes in separator electrode assembly microbial fuel cells. Bioresource Technology, 2013, 133: 74-81.

Zhang J, Zhang L. Influence of temperature on performance of anaerobic digestion of municipal solid waste. Journal of Environmental Sciences, 2006, 18(4): 810-815.

Zhang P, Chen Y, Zhou Q. Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: effect of pH. Water Research, 2009a, 43(15): 3735-3742.

Zhang L, Keller J, Yuan Z. Inhibition of sulfate-reducing and methanogenic activities of anaerobic sewer biofilms by ferric iron dosing. Water Research, 2009b, 43(17): 4123-4132.

Zhang T, Zeng Y, Chen S, et al. Improved performances of E. coli-catalyzed microbial fuel cells with composite graphite/PTFE anodes. Electrochemistry Communications, 2007, 9(3): 349-353.

Zhang Y, Sun J, Hu Y, et al. Bio-cathode materials evaluation in microbial fuel cells: a comparison of graphite felt, carbon paper and stainless steel mesh materials. International Journal of Hydrogen Energy, 2012, 37(22): 16935-16942.

Zhao Y, Boone D R, Mah R A, et al. Isolation and characterization of Methanocorpusculum labreanum sp. nov. from the LaBrea Tar Pits. International Journal of Systematic and Evolutionary Microbiology, 1989, 39(1): 10-13.

Zhao Z, Zhang Y, Chen S, et al. Bioelectrochemical enhancement of anaerobic methanogenesis for high organic load rate wastewater treatment in a up-flow anaerobic sludge blanket (UASB) reactor. Scientific Reports, 2014, 4: 6658.

Zhao Z, Zhang Y, Quan X, et al. Evaluation on direct interspecies electron transfer in anaerobic sludge digestion of microbial electrolysis cell. Bioresource technology, 2016, 200: 235-244.

Zhao Z, Zhang Y, Wang L, et al. Potential for direct interspecies electron transfer in an electric-anaerobic system to increase methane production from sludge digestion. Scientific Reports, 2015, 5: 11094.

Zheng M, Zheng M, Wu Y, et al. Effect of pH on types of acidogenic fermentation of fruit and vegetable wastes. Biotechnology and Bioprocess Engineering, 2015, 20(2): 298-303.

Zhou M, Chi M, Luo J, et al. An overview of electrode materials in microbial fuel cells[J]. Journal of Power Sources, 2011, 196(10): 4427-4435.

Zupancic G D, Roš M. Heat and energy requirements in thermophilic anaerobic sludge digestion. Renewable Energy, 2003, 28(14): 2255-2267.

Curriculum Vitae

Name	**Qing Feng**
Date of Birth	**November 16, 1988**
Place of Birth	**Heze, China**

Education

Ph.D. Department of Civil and Environmental Engineering
Korea Maritime and Ocean University, Busan, South Korea
September 2014 - August 2017
Advisor: Prof. Young-Chae Song, E-mail: soyc@kmou.ac.kr

M.S. Department of Civil and Environmental Engineering
Korea Maritime and Ocean University, Busan, South Korea
September 2012 - August 2014
Advisor: Prof. Young-Chae Song, E-mail: soyc@kmou.ac.kr

B.S. Department of Ocean Environment
Korea Maritime and Ocean University, Busan, South Korea
March 2008 - August 2012

Honor and awards

1. **Certificate of Scholarship**, Scholarship Foundation of East Asia Environment, Chungju, South Korea, November 10, 2016.
2. **Best Oral Presentation Award**, 11th Asia Biohydrogen and Biogas Symposium (ABBS 2016), Jeju, South Korea, October 8, 2016.
3. **Poster Session Gold Prize**, 4th Busan Golobal Water Forum, Busan, South Korea, Septermber 1, 2016.
4. **Certificate of Scholarship**, Busan Foundation for International Activities (BFIA), Busan, South Korea, April 3. 2015
5. **Certificate of Honor**, Consulate-General of the People's Republic of China in Busan, Busan, South Korea, December 31, 2012.

Publications

I. Thesis

1. "Bioelectrochemical Anaerobic Digestion for High-Rate methane production", Ph.D. Thesis, Korea maritime and Ocean University, August, 2017.
2. "Influence Factors on the Performance of Bioelectrochemical Anaerobic Digestion for Sewage Sludge", M.S. Thesis, Korea maritime and Ocean University, August, 2014.

II. International journal articles

1. **Qing Feng**, Young-Chae Song*, Yongtae Ahn, "Influence of anaerobic planktonic bacteria in bulk solution for electron transfer pathway in bioelectrochemical anaerobic digestion", Water Research, 2017, Submitted (Chapter 5).

2. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Nanthakumar Kuppanan, Sanjukta Subudhi, Banwari Lal, "Influence of neutralization in acidic

distillery wastewater on direct interspecies electron transfer for methane production in an upflow anaerobic bioelectrochemical reactor", International Journal of Hydrogen Energy, 2017, accepted (Chapter 6).

3. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Nanthakumar Kuppanan, Sanjukta Subudhi, Banwari Lal, "Bioelectrochemical enhancement of direct interspecies electron transfer in upflow anaerobic reactor with effluent recirculation for acidic distillery wastewater", Bioresource Technology, 2017, 241 (8) 171-180 (Chapter 6).

4. **Qing Feng** and Young-Chae Song*, "Decoration of graphite fiber fabric cathode with electron transfer assisting material for enhanced bioelectrochemical methane production", Journal of Applied Electrochemistry, 2016, 46(12):1211-1219 (Chapter 3).

5. **Qing Feng**, Young-Chae Song*, Byung-Uk Bae, "Influence of applied voltage on the performance of bioelectrochemical anaerobic digestion of sewage sludge and planktonic microbial communities at ambient temperature", Bioresource Technology, 2016, 220(6):500-508 (Chapter 4).

6. **Qing Feng** and Young-Chae Song*, "Surface modification of a graphite fiber fabric anode for enhanced bioelectrochemical methane production", Energy and Fuels, 2016, 30(8):6467-6474 (Chapter 3).

7. Young-Chae Song*, **Qing Feng**, Yongtae Ahn, "Performance of the bio-electrochemical anaerobic digestion of sewage sludge at different hydraulic retention times", Energy and Fuels, 2016, 30(1):352-359 (Chapter 4).

8. **Qing Feng**, Young-Chae Song*, Dong-Hyun Kim, Mi-Sun Kim, Dong-Hun Kim, "Bioelectrochemical enhancement of ambient temperature anaerobic digestion for sewage sludge", In preparation (Chapter 4).

III. Domestic journal articles

1. **Qing Feng**, Young-Chae Song*, Seong-Ho Jang, "Ferric chloride addition

enhances performance of bioelectrochemical anaerobic digestion of sewage sludge at ambient temperature", Journal of Korean Society Environmental Engineers, 2016, 38(11):618-626.

2. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Banwari Lal, Nanthakumar Kuppanan, Sanjukta Subudhi, Tae-Seon Choi, "Performance of upflow anaerobic bioelectrochemical reactor compared to the sludge blanket reactor for acidic distillery wastewater treatment", Journal of Korean Society Environmental Engineers, 2016, 38(6):279-290.

3. Dong-Hyun Kim, Young-chae Song*, **Qing Feng**, "Influence of applied voltage for bioelectrochemical anaerobic digestion of sewage sludge", Journal of Korean Society Environmental Engineers, 2015, 37(9):542-549.

IV. International conference proceedings

1. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Banwari Lal, Nanthakumar Kuppanan, Sanjukta Subudhi, "Electron Transfer pathway for Methane Production in Upflow Anaerobic Bioelectrochemical Reactor: Effect of Anaerobic Sludge Exposed to Electric Field", Proceeding of 2017 International Environmental Engineering conference (IEEC 2017), November 15-17, 2017.

2. Young-Chae Song*, **Qing Feng**, Dong-Hyun Kim, Seok-Oh Ko, "Performance of ambient temperature bioelectrochemical anaerobic digestion of sewage sludge compared to mesophilic condition", Proceeding of the 3rd International Conference on Environment and Renewable Energy (ICERE 2017), Ho Chi Minh, Vietnam, February 25-27, 2017.

3. Haenam Jang, **Qing Feng**, Byung-Uk Bae, Young-Chae Song*, "Effect of ferric ion on the bioelectrochemical anaerobic digestion of sewage sludge at ambient temperature", Proceeding of the 3rd International Conference on Environment and Renewable Energy (ICERE 2017), Ho Chi Minh, Vietnam, February 25-27, 2017.

4. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Banwari Lal, Nanthakumar Kuppanan, Sanjukta Subudhi, "Bioelectrochemical enhancement of Direct Interspecies electron transfer in UASB reactor for distillery wastewater", Proceeding of the 23rd Thailand-Korea Conference on Environmental Engineering, Bangkok, Thailand, February 5-9, 2017.

5. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Banwari Lal, Nanthakumar Kuppanan, Sanjukta Subudhi, "Effect of neutralization on the performance of upflow anaerobic bioelectrochemical reactor compared to the sludge blanket reactor for distillery wastewater treatment", Proceeding of the 11th Asia Biohydrogen and Biogas Symposium (ABBS 2016), Jeju, South Korea, October 5-7, 2016.

6. Nanthakumar Kuppanan*, Mrinalini Kumari, Pratiksha Jain, Sanjukta Subudhi, Banwari Lal, Young Chea-Song, **Qing Feng**, Kyuseon Yoo, "Pre-treatment of distillery wastewater for enhanced bio-electrochemical anaerobic methane production", Proceeding of the 3rd Asian Pacific Conference of the International Society for Microbial Electrochemistry and Technologies (AP-ISMET 2016), Busan, South Korea, August 31-September 2, 2016.

7. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Banwari Lal, Nanthakumar Kuppanan, Sanjukta Subudhi, "Effluent recirculation improves the performance of the upflow anaerobic bioelectrochemical reactor treating acidic distillery wastewater", Proceeding of the 4th Busan Global Water Forum, Busan, South Korea, August 31-September 1, 2016.

8. **Qing Feng**, Young-Chae Song*, "Effect of temperature and ferric ion on the bioelectrochemical anaerobic digestion of sewage sludge", Proceeding of the 22nd Korea-Thailand Conference on Environmental Engineering, Daegu, South Korea, April 20-23, 2016.

9. Yongtae Ahn, Dong-Hyun Kim, **Qing Feng**, Jae-Woo Chung, Si-Kyung Cho, Young-Chae Song*, "Bioelectrochemical Anaerobic Digestion for sewage sludge at different low applied voltage", Proceeding of the 2nd

International Conference on Environment and Renewable Energy (ICERE 2016), Ho Chi Minh, Vietnam, February 24-26, 2016.

10. Young-Chae Song*, **Qing Feng**, Donghyun Kim, "Effect of different anodes on the enhanced methane production from bioelectrochemical anaerobic digester", Proceeding of International Environmental Engineering Conference (IEEC 2015), Busan. South Korea, October 28-30, 2015.

11. Young-Chae Song*, **Qing Feng**, "Effect of temperature on the performance of bioelectrochemical anaerobic digestion for sewage sludge", Proceeding of the 10th Asia Biohydrogen and Biogas Symposium (ABBS 2015), Keelung. Taiwan, October 14-16, 2015.

12. Young-Chae Song*, **Qing Feng**, "BioElectrochemical Anaerobic Digestion for methane from sewage sludge", Proceeding of the 21st Thailand-Korea Conference on Environmental Engineering, Chiengmai. Thailand, February 2-7, 2015.

13. Young-Chae Song*, **Qing Feng**, Yongtae Ahn, "Bioelectrochemical anaerobic digestion for methane from sewage sludge", Proceeding of Biological-Waste as Resource, with a Focus on Food Waste, Hong Kong, China, December 1-3, 2014.

V. Domestic conference proceedings

1. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, Nanthakumar Kuppanan, Sanjukta Subudhi, Banwari Lal, "The Influence of Electrode Placement on the Upflow Anaerobic Bioelectrochemical Reactor for Distillery Wastewater Treatment", Proceeding of Korea Society of Waste Management, Jeonju, South Korea, May 10-12, 2017.

2. Young-Chae Song*, **Qing Feng**, "Bioelectrchemical enhancement of dIrect interspecies electron transfer for high rate anaerobic digestion", Proceeding of Korea Society of Waste Management, Gwangju, South Korea, March 23-24, 2017.

3. **Qing Feng**, Young-Chae Song*, Dong-Hyun Kim, "The influence of applied voltage method and ferric ion on the bioelectrochemical anaerobic digestion", Proceeding of Korea Society of Environmental Engineers, Gyeongju, South Korea, November 16-18, 2016.

4. **Qing Feng**, Young-Chae Song*, Kyuseon Yoo, "Anaerobic bioelectrochemical process compared to UASB for treatment of distillery wastewater", Proceeding of Korean Society on Water Environment, Busan, South Korea, March 23-25, 2016.

5. Young-Chae Song*, **Qing Feng**, "Effect of HRT on the performance of bioelectrochemical anaerobic digestion at ambient temperature", Proceeding of Korea Organic Resources Recycling Association, Seoul, South Korea, January 8, 2016.

6. Young-Chae Song*, **Qing Feng**, Kyuseon Yoo, "Effect of reduction catalysts on the enhanced methane production from bioelectrochemical anaerobic digester", Proceeding of Korean Society on Water Environment, Goyang, South Korea, August 26-28, 2015.

7. Young-Chae Song*, **Qing Feng**, "Performance of bioelectrochemical anaerobic digestion for sewage sludge at ambient temperature", Proceeding of Korea Society of Waste Management, Daejeon, South Korea, May 21-22, 2015

8. Young-Chae Song*, **Qing Feng**, "Effect of potential difference between anode and cathode on the low temperature BioElectrochemical Anaerobic Digestion for sewage sludge", Proceeding of Korea Organic Resources Recycling Association, Seoul, South Korea, May 7, 2015.

9. Young-Chae Song*, **Qing Feng**, "Effect of temperature on the performance of bioelectrochemical anaerobic digestion for sewage sludge", Proceeding of Korea Organic Resources Recycling Association, Seoul, South Korea, November 28, 2014.

10. Young-Chae Song*, **Qing Feng**, "Performance of bioelectrochemical

anaerobic digestion for Purified terephthalic acid(PTA) wastewater treatment", Proceeding of Korea Society of Waste Management, Gyeongju, South Korea, November 13-14, 2014.

11. Young-Chae Song*, **Qing Feng**, "Effect of applied voltage on the performance of bioelectrochemical anaerobic digestion for sewage sludge", Proceeding of Korea Society of Environmental Engineers, Gwangju, South Korea, August 21-22, 2014.
12. Young-Chae Song*, **Qing Feng**, "Effect of Hydraulic Retention Time on the Performance of Methane-producing Microbial Electrolysis Cell running on sewage sludge", Proceeding of Korea Society of Waste Management, Busan, South korea, May 15-16, 2014.
13. Young-Chae Song*, **Qing Feng**, "Carbon Nanotube coating on Graphite Fiber Fabric via Electrophoresis Deposition for electrode of Microbial Electrolysis Cells", Proceeding of Korea Society of Waste Management, Jeju, South Korea, November 14-16, 2013.

VI. Research project

1. "A collaborative study on the novel high-rate bioelectrochemical anaerobic digestion process for renewable energy recovery from distillery waste water technology", National Research Foundation of Korea, South Korea, 2015.05.01-present.
2. "Study on the high rate bioelectrochemical anaerobic digestion for sewage sludge", National Research Foundation of Korea, South Korea, 201.11.01-present.
3. "Developing Anaerobic digestion to generate methane gas by using bioelectrochemical technology", Korea Institute of Energy Research, South Korea, 2015.01.01.-2015.10.31.
4. "Development of practical technology for high efficiency microbial fuel cell", Korea Institute of Energy Technology Evaluation and Planning, South Korea, 2013.06.24.-2014.06.23.